Quantifying environmental responses of terrestrial plant communities

Thomas M.W.J. van Goethem

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Thomas Matthijs Wilhelmina Josephus van Goethem

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Promotor:	Prof. Dr. M.A.J. Huijbregts
Copromotores:	Dr. A.M. Schipper
	Dr. R. van Zelm
Leden manuscriptcommissie:	Prof. dr. H. de Kroon (voorzitter)
	Prof. dr. L.P.M Lamers
	Prof. dr. ir. P.M van Bodegom (CML Leiden)
Paranimfen:	Lisette de Hoop
	Nils van Rooijen

- Patience is the art of hoping -

Marquis de Vauvenargues

Contents

Chapter 1

General Introduction

1.1 Background

Species sensitivity distributions

Species assemblages are typically shaped by multiple environmental factors (Soberón, 2007; Schipper et al., 2014a). Quantifying the relationship between species (communities) and the abiotic environment provides valuable information to protect natural systems. The species sensitivity distribution (SSD) approach may prove useful to quantify the relationships between species and specific environmental stressors (Van Straalen & Denneman, 1989; Posthuma et al., 2002). SSDs have been developed for various toxic compounds and are generally applied in ecotoxicology (De Hoop et al., 2011; Fedorenkova, 2015). Typically, SSDs for toxicants are cumulative distributions of laboratory-derived toxicity data for a single chemical (Raimondo et al., 2007; Posthuma & De Zwart, 2012). Species survival, fecundity or growth is often used as endpoint indicator in these exposure experiments (Zeeman et al., 1999; Suter, 1998).

SSDs can be applied to set environmental quality standards (EQS) and to assess environmental effects in a geographical and product context (Cardwell et al., 1999; Posthuma et al., 2002). These applications are further explained below (see also Figure 1.1).

Environmental quality standards

EQS are set with the aim to protect the environment from damage by anthropogenic environmental stressors. In an ecological context, an EQS typically refers to the level of the environmental stressor that is protective of a predefined proportion of the species pool, usually 95% (Kefford et al., 2011; Cormier & Suter II, 2013). If the EQS is exceeded, it is assumed that the stressor poses a risk to the ecosystem of interest that requires further consideration (Posthuma et al., 2002). Environmental quality standards based on SSDs are typically derived for toxic chemicals released from anthropogenic sources in aquatic ecosystems (Fedorenkova et al., 2015).

Environmental impact assessment

Environmental impact assessments (EIA) are performed to assess the extent to which an ecosystem is affected by the level of the environmental stressor occurring in that location (Van Straalen & Denneman, 1989; Posthuma et al., 2002). SSDs can be used to estimate the potentially affected fraction (PAF) of species exposed to one or more

environmental stressors (Klepper et al., 1998; De Zwart and Posthuma, 2006). SSDs are typically applied for EIA of toxic compounds in freshwater, soil and marine ecosystems (Fedorenkova et al., 2015).

Life cycle impact assessment

SSDs can also be used in life cycle impact assessments (LCIA), in order to quantify the environmental impacts associated with the production, use or disposal of products (Consoli et al., 1993; Van Zelm, 2011). In LCIA a weighted summation of emissions is done with help of so called characterization factors (CFs). CFs represent the environmental impact of a pollutant per unit of emission (Udo de Haes et al., 2002). A CF consists of a fate factor (FF), quantifying the relationship between the emission of a pollutant and the exposure concentration at the receiving location, and an effect factor (EF), quantifying the environmental impact following an increase of the pollutant concentration at the receiving location. SSDs are used to determine the EF, for instance, by quantifying the marginal change in the impact due to the marginal change in the exposure to a pollutant at the receiving location.

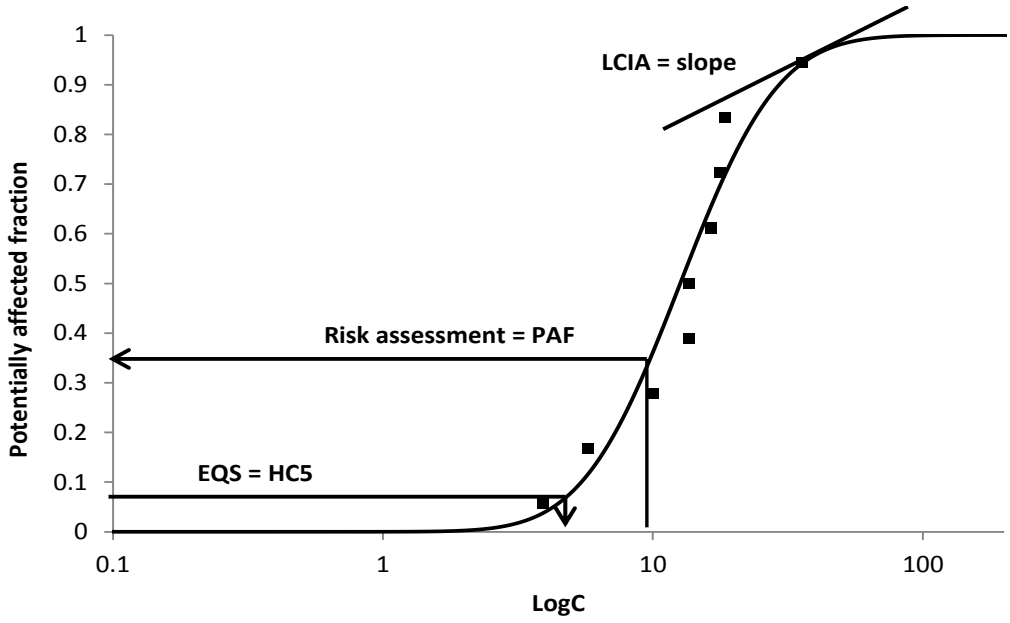

Figure 1.1 Schematic representation of an SSD showing the potentially affected fraction (PAF) of species in relation to the level (concentration) of an environmental stressor (logC). The dots are observations and the line is a fitted SSD. An SSD can be used to set an EQS (here the hazardous concentration for 5% of the species, HC_5), to assess environmental risk (here the PAF corresponding with the concentration at a given location) or to derive a marginal effect factor used in LCIA (here the slope of the curve at the background concentration for a receiving location).

1.2 Problem setting

SSDs based on laboratory experiments have several limitations. First, these SSDs are generally derived from tests with a few easily cultured aquatic species that may be absent from the field site of concern. Because species may differ strongly in their sensitivity to environmental stressors, these SSDs are not necessarily ecologically representative (Kefford et al., 2012). Second, SSDs for non-toxic stressors in the terrestrial environment are mostly lacking, with the exception of a few studies that developed SSDs for acidification (Azevedo et al., 2013; Wamelink et al., 2005; Wamelink et al., 2012). Species assemblages, however, are typically shaped by multiple (non-toxic) environmental factors (Soberón, 2007; Schipper et al., 2014a). Third, endpoints used in SSDs often reflect an affected fraction of species based on survival, fecundity or growth (Zeeman et al., 1999; Suter, 1998). Endpoints on the community level, for instance reflecting taxonomic or functional diversity, are typically lacking, while they may add valuable information on the effect of environmental stressors on ecosystems (Brenson et al., 1993; Pastorok et al., 2002).

1.3 Opportunities and challenges

Here, several approaches are proposed to address the limitations of the SSD methodology as described in section 1.2. The potential benefits and challenges that come with the proposed expansion of the SSD approach are addressed below.

Representative species

It is important to derive SSDs based on a representative species pool as species can differ strongly in their sensitivity to environmental stressors (Azevedo, 2014; Posthuma et al., 2002). SSDs can also be derived for specific species groups when data is available for more representative species. These SSDs can be used to assess differences in sensitivity between different species groups (Leuven et al., 2011; Azevedo et al., 2013; Verbrugge et al. 2012). Such SSDs might provide an important tool for protecting endemic, keystone or commercially valued species (Azevedo, 2014; Fedorenkova, 2015).

Non-toxic environmental factors

SSDs for non-toxic environmental stressors have the potential to provide complementary information in environmental assessments based on indicator species and expert judgment (Ashmore, 2005). SSDs for non-toxic stressors have also added

value for application in LCIA, as it has been concluded that impact assessments for non-toxic environmental stressors, particularly in the terrestrial environment, need improvements (Van Zelm, 2011). In this context, tropospheric ozone formation, acidification and eutrophication are considered as the three main drivers determining the community composition of terrestrial vegetation (Wamelink et al., 2012; Schaminée et al., 1995; Ashmore, 2005).

Novel endpoints

With the SSD approach, effects of environmental stressors on species assemblages are typically quantified based on taxonomic richness. It is, however, increasingly emphasized that the number of species alone is not fully representative of the diversity of species communities (Villéger et al., 2008; Mori et al., 2013). Functional diversity reflects the values and ranges of the functional traits of the species in an assemblage. As traits influence species' performance, functional diversity measures are considered particularly relevant from the perspective of ecosystem functioning (Diaz & Cabido, 2001). Up till now, however, very few studies have derived SSDs with functional diversity as endpoint (Mason et al. 2012). SSDs based on assemblage-level characteristics other than species richness provide additional information on the ecological effects of environmental stressors (Mason et al., 2011; Mouchet et al., 2010).

SSDs based on field data

Deriving SSDs from field data has recently been proposed as an alternative to lab-based SSDs (Azevedo et al., 2014; Kefford et al., 2012; Struijs et al., 2005). Field-based SSDs (f-SSDs) have the advantage to include the relevant species pool and environmental factors. It is, however, not straightforward to extract relationships between species richness and single factors from field data, as confounding environmental factors generally result in considerable scatter among species richness observations (Cade & Noon, 2003; Van den Brink et al., 2002). Various approaches to derive field-based SSDs (f-SSD) have been proposed (Schipper et al., 2014b). One method is to relate observations of taxonomic richness to a corresponding environmental variable, for example with quantile regression (Crane et al., 2007; Iwasaki & Ormerod, 2012). Quantile regression based on one of the upper boundaries of the response variable distribution (e.g. the 0.95 quantile) is expected to show the constraints imposed by the explanatory environmental variable of concern (Iwasaki & Ormerod, 2012).Alternative approaches use occurrence data to either calculate the number of species within regular

intervals along a particular environmental gradient (Kefford et al., 2011; Struijs et al., 2011), or to establish species-specific tolerance ranges or occurrence thresholds, which are then stacked across the species (Verbrugge et al., 2012; Azevedo et al., 2013; Cormier et al., 2013). These approaches, however, have not yet been quantitatively compared with each other. Differences in the f-SSDs resulting from the various approaches (magnitude and width of the response curves) can lead to differences in EQS and environmental risk predictions (Struijs et al., 2011). Information on the similarities and differences of the approaches can help to determine the appropriate applicability domain.

1.4 Aim

The aim of this thesis was to develop and apply SSDs to quantify the effects of ozone formation, acidification and eutrophication on taxonomic and functional characteristics of terrestrial plant communities. The SSDs developed are aimed (1) to cover a representative species pool, (2) to include more (relevant) environmental factors, and (3) to apply novel, assemblage-level endpoints, as compared to the current state of the art.

Terrestrial plant communities were selected because they are the foundation for most terrestrial ecosystems. Furthermore, vegetation is a suitable study object as plants are, mainly, sessile organisms. They can therefore be directly linked to habitat conditions. Moreover, large datasets are available with field observations of species occurrence and abundance and measurements of the relevant environmental stressors and with plant traits (Schaminée et al., 1995; Wamelink et al., 2012).

1.5 Thesis outline

Table 1.1 provides an overview of the SSDs developed and applied in the different thesis chapters. In chapter 2, the effect of tropospheric ozone was assessed for three species groups, i.e. trees, annual grassland species and perennial grassland species. The effect was assessed by deriving SSDs based on species-specific response data obtained from laboratory experiments. The SSDs were applied to both derive critical ozone levels and estimate the potentially affected species fraction of plant communities in Northwestern Europe along specific ozone gradients.

The SSDs for tropospheric ozone were applied in the context of life cycle impact assessment in chapter 3. Here, the impact of tropospheric ozone on natural vegetation caused by anthropogenic emissions of NOx and NMVOC was estimated. The impact was calculated for 65 European regions.

In chapter 4, three methods were compared to derive f-SSDs and corresponding EQS: quantile regression, species accumulation curves and stacked species occurrence ranges. The methods were applied to a common dataset with plant species observations and measured pH values in grasslands.

In chapter 5, f-SSDs and EQS were derived for soil nitrate conditional on soil pH. Again, quantile regression was used. The dataset contained measured pH and NO_3 values and species-specific field observations in grasslands and forests.

In chapter 6, taxonomic and functional diversity were quantified in relation to a pH gradient for semi-natural grassland communities. Taxonomic and functional diversity indicators were related to pH with quantile regression based on the same dataset as used in chapter 4. The resulting environmental response curves were compared and interpreted in the context of community assembly processes.

Finally, in chapter 7, the results of the preceding chapters were used as a basis to discuss the benefits and limitations of field- and laboratory-based SSDs, and address implications and recommendations for future research.

Table 1.1. Overview of the species sensitivity distributions developed and applied per chapter

Chapter	Stressor	Data type	Aggregation level	Endpoint	Vegetation type	Application
2, 3	Tropospheric ozone	Laboratory	Species	Potentially affected fraction	Grasslands, forests	EQS and ERA (2), LCIA (3)
4, 6	Soil pH	Field observations	Species, Community	Taxonomic and functional diversity	Grasslands	EQS (4), ecological theory (5)
5	Nitrate	Field observations	Community	(relative-) Species richness	Grasslands, forests	Context dependent EQS

Chapter 2

Plant species sensitivity distributions for ozone exposure

Thomas M.W.J van Goethem

Ligia B. Azevedo

Rosalie van Zelm

Felicity Hayes

Mike R. Ashmore

Mark A. J. Huijbregts

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Abstract

This study derived Species Sensitivity Distributions (SSD), representing a cumulative stressor-response distribution based on single-species sensitivity data, for ozone exposure on natural vegetation. SSDs were constructed for three species groups, i.e. trees, annual grassland and perennial grassland species, using species-specific exposure-response data. The SSDs were applied in two ways. First, critical levels were calculated for each species group and compared to current critical levels for ozone exposure. Second, spatially explicit estimates of the potentially affected fraction of plant species in Northwestern Europe were calculated, based on ambient ozone concentrations. We found that the SSD-based critical levels were lower than for the current critical levels for ozone exposure, with conventional critical levels for ozone relating to 8-20% affected plant species. Our study shows that the SSD concept can be successfully applied to both derive critical ozone levels and estimate the potentially affected species fraction of plant communities along specific ozone gradients.

Key words: Ozone; Ecological Risk Assessment; AOT40; Species Sensitivity Distribution; Potentially Affected Fraction

2.1 Introduction

Northern Hemisphere tropospheric background ozone concentrations have increased over recent decades, as peak concentrations have fallen in North America and Europe (Derwent et al. 2007; Vingarzan, 2004). Background concentrations are predicted to further increase with 0.5 – 2% per year over the next 50 years primarily due to elevated emissions of nitrogen oxides and volatile organic compounds (Emberson et al., 2003; Royal Society, 2008). The adverse effects of ozone pollution on plants, including trees and grassland species, are of considerable concern (Emberson et al. 2007; Mills et al., 2007a, b). Some of these effects include growth and seed production reduction (Booker et al., 2009), premature senescence (Tonneijck et al., 2004), reduced ability to withstand stressors (Wilkinson and Davies, 2009), and an increase in leaf injury (Manning et al., 2002).

Critical levels are based on relationships between ozone concentrations and effects such as yield loss and biomass reduction (Hayes et al., 2006; Pleijel et al., 2007; Tuovinen et al., 2007). These levels are expressed as an Accumulated exposure Over a Threshold of 40 ppb (AOT40) and are based on sensitive but ecological relevant species (LRTAP, 2010, Matyssek et al. 2007). These species, and corresponding critical levels, are used as indicators to determine the risk for species groups or plant communities (Musselman and Lefohn, 2007). For example, critical levels of *Trifolium sp*. are assumed representative for all species of the productive grassland community (Klingberg et al., 2011). For monoculture arable crops and productive trees, such an approach of defining a critical level based on a single species for that community is possible. However, for semi-natural plant communities, with the large range of species present, an approach based on a single indicator such as *Trifolium* ignores the wide range of sensitivity across all the component species (Hayes et al., 2007; Mills et al. 2007b). To date, an approach which gives the affected fraction of a species assemblage due to ozone exposure is lacking in risk assessment for semi-natural vegetation (Ashmore, 2005; Paoletti and Manning, 2007).

In contrast, in most areas of ecotoxicology, Species Sensitivity Distributions (SSDs) are used (1) to derive environmental quality objectives of chemicals set equal to the concentration at which 5% of the species are affected (HC_5), and (2) to estimate the fraction of species affected at different exposure concentrations of chemicals (Posthuma et al., 2002). An SSD is a cumulative distribution of responses of different biological

species to the same stressor (Van Straalen et al., 1989). The SSD concept is a standard approach in ecotoxicology which is applicable to ozone risk assessment. It offers opportunities to both derive critical levels and estimate the affected fraction of species within a plant community along a specific ozone gradient.

The goal of this study was to develop SSDs for ozone exposure on natural vegetation. Our study includes 96 plant species. SSDs were constructed from species-specific ozone-response data provided by a comprehensive review of scientific literature and databases. Species were grouped according to response type (decrease or no decrease of biomass) and taxonomy (trees, annual and perennial grassland species). Critical threshold levels for ozone based on HC_5 were compared with AOT40-based critical levels commonly used in environmental policy assessment for ozone exposure. Finally, we show how the SSDs can be applied in practice by deriving spatially explicit estimates of potentially affected fraction of plant species in Northwestern Europe.

2.2 Methods

In order to derive SSDs, we first gathered species-specific ozone exposure-response functions from the literature. In these functions the measure of ozone exposure was expressed as AOT40, calculated as the sum of the differences between the hourly mean ozone concentration (in ppb) and 40 ppb during daylight hours. The exposure-response functions were used to calculate for each species the AOT40 value related to a 10% effect (EC_{10}). These species-specific EC_{10} values were subsequently used to derive the average and standard deviation of the SSD for each vegetation type. The steps from gathering species-specific data on ozone effects and acquiring SSDs to deriving HC_5 - values are described below.

Data gathering

Data on the effects of ozone concentrations on plants were collected from peer-reviewed studies published up to April 2012. The following keywords were used in the Boolean search (incl. keyword extensions) in Web of Science: (1) ozone; and (2) either vegetation, plant, tree, grassland; and (3) either critical levels, dose-response relationship, exposure, response, biomass; and (4) either open top chamber (OTC), AOT40, Free-Air Concentration Enrichment (FACE), exposure based model. This literature search provided 980 peer-reviewed studies to be considered. In addition to the Boolean search we used the data from the OZOVEG database (Hayes et al., 2007).

Data selection

Following Mills et al. (2007a) and Hayes et al. (2007), ozone exposure-response data from individual species were only included when the following criteria were met:

(1). It should not be a factorial experiment, testing for the effect of a treatment variable in addition to ozone, e.g. CO2 + O3 exposure, except when the specific effect of ozone without the treatment variable could be quantified.

(2) Experiments should be conducted under 'close to field' conditions, either using an open-top chamber (OTC), field release system (e.g. Eastburn, 2006) or solardome (e.g. Rafarel et al., 1995).

(3) The accumulated exposure above the critical 40ppb level should be at least be 21 days to ensure chronic exposure.

(4) The mean ozone concentration for any hour of the day should be maximum 100 ppb to take only realistic field conditions into account.

(5) Only ozone response data for individual species and not higher taxonomic groups (e.g. family, class, etc.) were considered. An exception was made for genus-level records in case no other species belonging to that particular genus was listed.

(6) Experiments should report the change in biomass. This endpoint is commonly used for ozone risk assessment in plants (LRTAP, 2010).

Ozone exposure-response relationships were found for a total of 96 species. For grassland species functions available from the OZOVEG database, along with new data for the additional species were used (Hayes et al., 2007), for trees data presented in Calatayud et al. (2011), Karlsson et al. (2003), Karlsson et al. (2004), Landolt et al. (2000), Skärby et al. (2004) was used.

Data handling

First, species synonyms were excluded using The Plant List (2010) to avoid double counting of species names. The effects of ozone on biomass were calculated relative to the charcoal-filtered air treatment (or occasionally non-filtered air if no charcoal filtered control was used). EC_{10} values were then calculated using the standardized dose-

response functions. Species exhibited two types of response when exposed to ozone, either biomass reduction (negative slope) or no biomass decrease (positive slope). The linear functions for biomass decrease were converted as follows:

$$EC_{10} = \frac{-0.1 \cdot b}{a} \tag{1}$$

, where b is the intercept and a is the slope of the linear function.

A list of all species with their dose-response functions and EC_{10} values can be found in the Supplementary information (S1, S2 and S3).

Species sensitivity distributions

Species Sensitivity Distributions (SSDs) were developed for three separate groups of species, i.e. trees, annual grassland species and perennial grassland species. For each group there were two effect definitions:

- one SSD was derived based on EC_{10} values for biomass reduction only;
- one SSD was derived for biomass reduction, corrected for the fraction of species with no biomass reduction (f_{nbd}).

SSDs were derived in the following way. First the EC_{10} data were log-transformed. Second, the mean (μ) and standard deviation (σ) of the log EC_{10}-data were calculated. Assuming a lognormal SSD for ozone exposure, the parameters μ and σ were then used to derive the Potentially Affected Fraction (PAF):

$$PAF = \frac{a}{\sigma \cdot \sqrt{2 \cdot \pi} \cdot AOT40 \cdot \ln 10} \cdot \int_0^{AOT40} \exp\left(-\frac{1}{2} \cdot \left(\frac{\log(AOT40) - \mu}{\sigma} \right)^2 \right) dAOT40 \tag{2}$$

, where *a* is 1 for the SSD derived based on EC_{10} values for biomass reduction only and *a* equals 1- f_{nbd} for the SSD derived including the fraction of species with no biomass reduction. AOT40 represents the ambient ozone exposure.

Differences in sensitivity between the species groups were investigated by comparing the means (μ) and variances (σ). The log10-transformed EC_{10} values were tested for normality with the Kolmogorov Smirnov test. The means were compared with the

Independent t-test and the variances (σ) were compared using the Levene's test. All tests were executed with SPSS 17.0 for Windows.

Critical levels

Hazardous exposure concentrations for which 5% of the species assemblage remains unprotected (HC_5) were derived for each species groups and their respective response types. The HC_5 for the species with biomass reduction only was calculated following the procedure described by Aldenberg and Jaworska (2000):

$$LogHC_5 = \mu - k \cdot \sigma \quad (3)$$

where k is the extrapolation constant for 95% species protection. Aldenberg and Jaworska (2000) present extrapolation constants for the estimation of the log(HC_5) based on the assumption of normal species sensitivity distributions for the log-transformed toxicity data. To assess the uncertainty of the HC_5 the 90% confidence interval was calculated following Aldenberg and Jaworska (2000).

The HC_5 for the species assemblage including the fraction of species with no biomass reduction was derived by calculating the concentration at which $5/(1-f_{nbd})$% of the sensitive species is affected.

PAF levels corresponding to the critical levels recommended by the LRTAP Convention (2010) were determined using the lognormal SSD function. The 90% confidence interval was calculated following methods adapted from Aldenberg and Jaworska (2000).

Impact assessment

Maps of the potentially affected fraction (PAF) of species were compiled to determine the impact of ozone exposure on annual and perennial grassland species in Northwestern Europe. A spatially explicit grid-based approach on a 0.5 x 0.5 degree (i.e. ca. 50km x 50km at 60º N) resolution was applied. Grid-specific AOT40 exposure concentrations for 2010 were obtained using the EMEP model (Jonson et al. 2001). The AOT40 values were based on a growing season of May-July at a height of 1m above the ground. In each grid the PAF was derived for each species groups using the AOT40 exposure values as input in the SSD (equation 3).

2.3 Results

Species sensitivity distributions

Exposure-response functions were determined for 25 annual grassland species, 62 perennial grassland species, and 9 tree species. The full data set is given in the SI (tables S2.1, S2.2 and S2.3). The percentage of species in the dataset that exhibited a biomass reduction was 88% for annual grassland species, 63% for perennial grassland species and 100% for tree species. According to the Kolmogorov Smirnov test all EC_{10}-data were normally distributed.

Figure 2.1 shows the species sensitivity distributions for annual grassland species, perennial grassland species and trees based on EC_{10}-data (a) and with the fraction of species with no biomass decrease included (b). Significant differences in means were found for annual and perennial grassland species, i.e. $p = 0.01$ for biomass reduction. Significant differences in variances were found for annual grassland species and trees. All results of the statistical testing of differences in means and variances can be found in the SI (S2.4).

Critical levels

HC_5 values varied from 1.3 to 4.1 ppm.h for the various species groups and effect definitions with no statistically significant differences (Table 2.1). The HC_5 values for annual and perennial grassland species were consistently lower than the corresponding critical levels. The PAFs relating to the current critical levels were derived for each species group. These indicated that potentially 8% of tree species, 17% of perennial grassland species, and 20% of annual grassland species have a growth reduction of at least 10% due to ozone exposure at the current critical level.

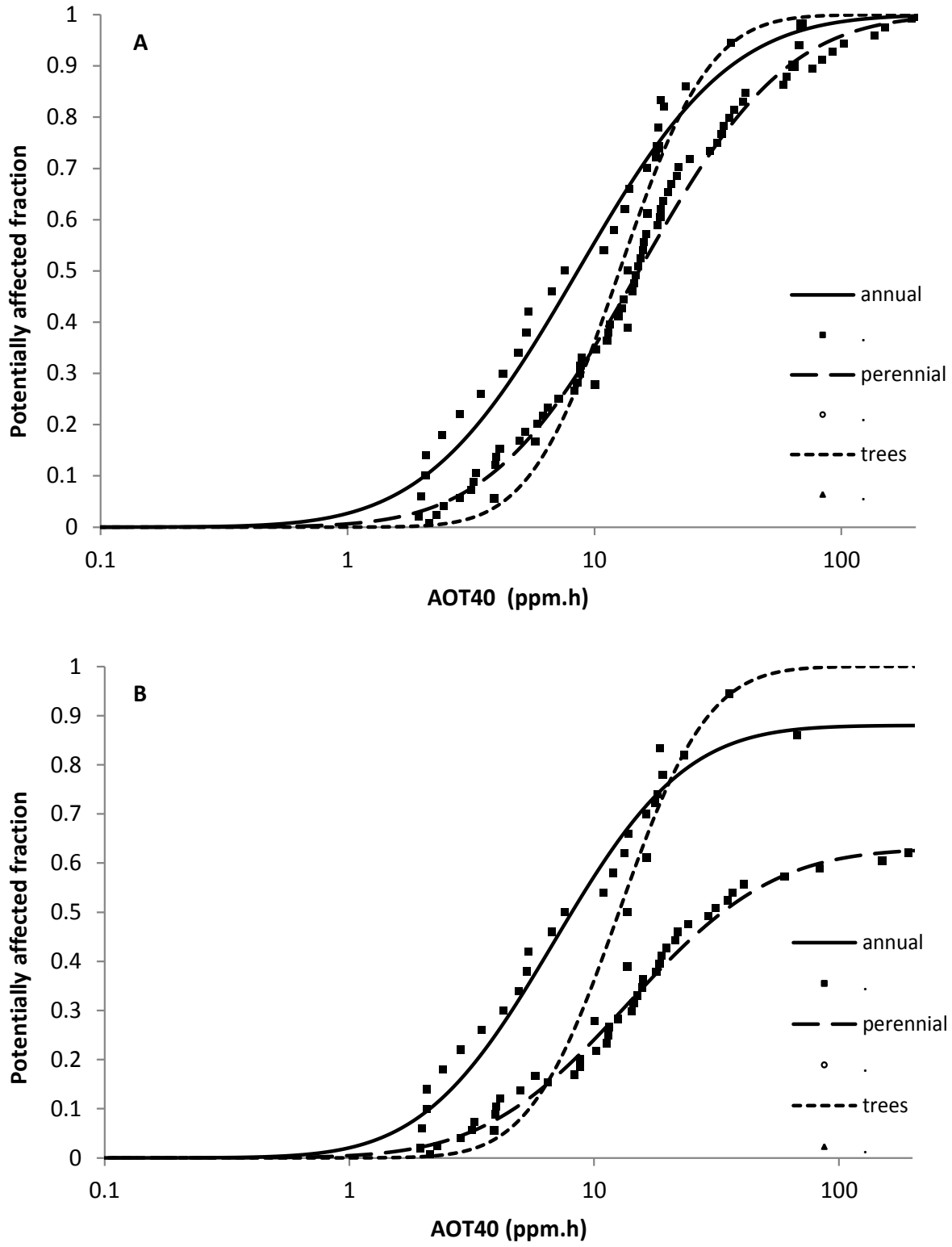

Figure 2.1. Species sensitivity distributions for annual grassland species (solid line), perennial grassland species (dotted line) and trees (finely dotted line) based on biomass reduction only (a) and with the fraction of species with no biomass decrease included (b).

Table 2.1. Means (μ) and standards deviations (σ) of HC_5 for trees, annual grassland species and perennial grassland species, based on EC_{10}-data for the individual species within the group, HC_5 values in ppm.h (90% confidence interval) and PAF values corresponding to the critical level (90% confidence interval).

		n species	μ	σ	HC_5	Critical level[1]	PAF calculated for current critical levels of ozone
Annual grassland species	Biomass reduction only	22	0.84	0.42	1.37 (0.75-2.09)	3	0.20 (0.10-0.28)
	Fraction no biomass decrease	25	0.84	0.42	1.67 (0.81-2.58)	3	0.17 (0.09-0.30)
Perennial grassland species	Biomass reduction only	39	1.14	0.47	2.33 (1.59-3.19)	5	0.17 (0.09-0.30)
Trees	Fraction no biomass decrease	62	1.14	0.47	2.81 (1.77-4.13)	5	0.11(0.06-0.21)
	Biomass reduction only	9	1.10	0.29	4.10 (1.72-6.58)	5	0.08 (0.01-0.28)

[1]Critical levels based on the AOT40-based method determined by LRTAP convention 2010.

Impact assessment

The actual PAF of grassland species, calculated based on modeled ozone concentrations in Northwestern Europe is shown in Figure 2.2 on a 0.5x0.5 degree grid level. PAF values varied between 0.00-0.30 for different species groups and effect definitions. The values indicate that in some regions potentially 13% of the perennial grassland species and 30% of annual grassland species have growth reductions of at least 10% when exposed to ambient ozone concentrations equivalent to those of 2010. From these maps it can be seen that continental Europe has the highest PAFs.

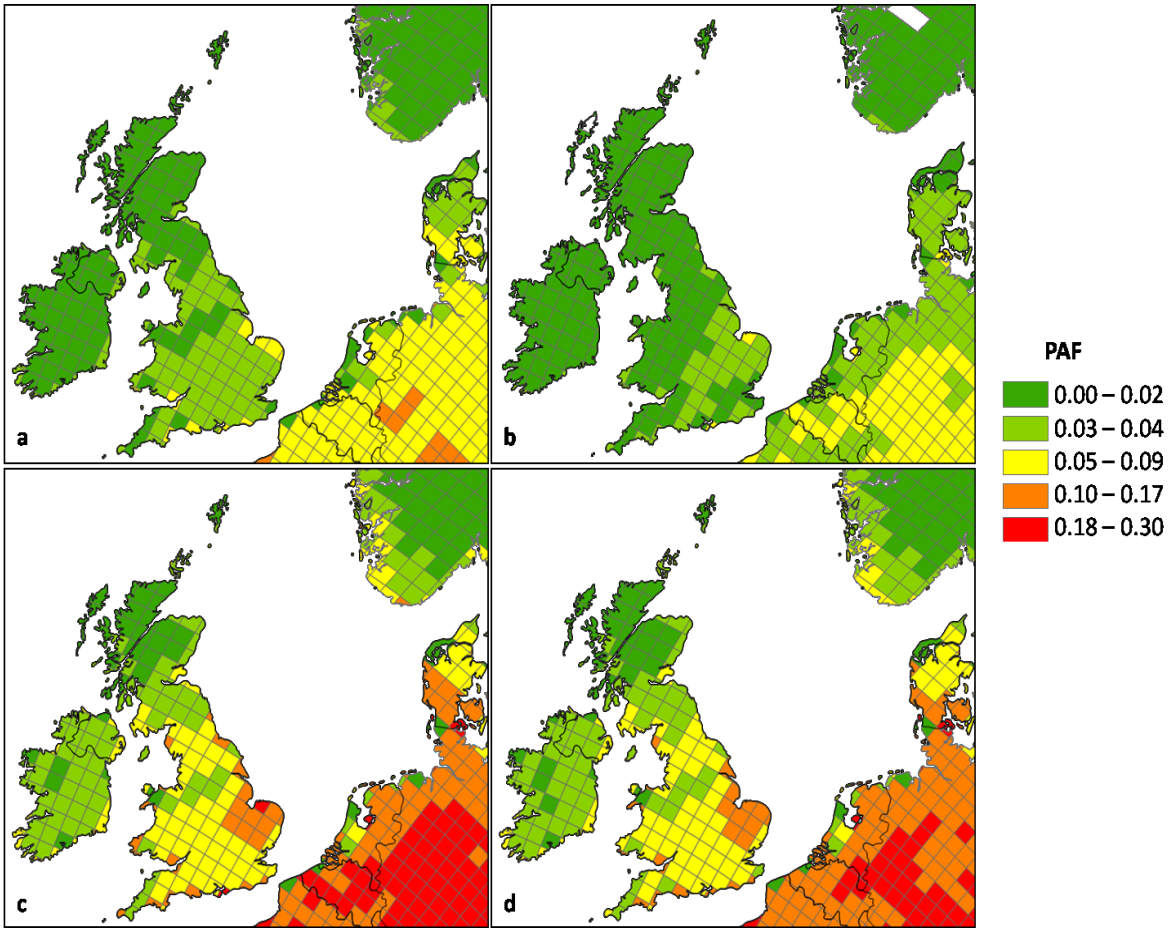

Figure 2.2. The potential affected fraction corresponding to modeled ozone levels (AOT40 in 2010) for perennial grassland species using biomass reduction only (a) and including the fraction of species with no biomass decrease (b), and for annual grassland species using biomass reduction only (c) and including the fraction of species with no biomass decrease (d).

2.4 Discussion

We derived SSDs for effects of ozone exposure on natural vegetation. Species were grouped according to endpoint (biomass decrease or no decrease) and taxonomy (trees, and annual and perennial grassland species). Both critical levels and spatially explicit impacts were determined. In the following, we discuss the main factors driving uncertainties regarding the AOT40-based effect data and extrapolation of data. After that, the results are interpreted and the application of SSDs in ozone risk assessment is discussed.

Uncertainties

Here, the concentration-based AOT40 method was used to estimate the risk of damage by ozone to natural vegetation. The use of the time integrated AOT40 index could lead to biases when the duration of exposure is very different from the model context where it is applied. In our study, however, the exposure duration and the modeled range of AOT40 are in line with each other. We used linear response models to describe species-specific ozone effect relationships. Such relationships are generally reported for crops in open top fumigation experiments (Musselman et al., 2006). However, for trees and semi-natural grassland communities non-linear response models have also been used to describe ozone exposure-effect relationships (Fuhrer et al., 1997; Manes et al., 2005). In particular, some studies have shown that perennial plants can have a non-linear response to long term ozone exposure of >2 yrs (Matyssek et al. 2003). These effects, however, are not yet fully understood because most fumigation experiments run for only 1 growing season (Kitao et al. 2009). Nevertheless, we have chosen to use linear exposure-response functions to determine our EC_{10} values because of the availability of data. The species-specific exposure-response relationships were directly taken from the literature and the number of data points in the published regressions differed widely between the species involved (3 to 145, 7 on average). A number of regressions have low R2 values for perennial and annual grassland species. As a sensitivity check, we derived HC_5 values only using species response curves with respectively $R2 > 0.5$ and $R2 > 0.75$ as cut off criteria (table S5). We found that the HC_5 values for the subselection of species with relatively high R2 values are not statistically different from the HC5 values based on all species information Moreover, some functions were based on a single experiment, hereby leading to an over- or underestimation of the response of individual plants to ozone. Furthermore, it is not known how representative exposure-response relationships determined in fumigation experiments using tree seedlings or saplings are for mature trees. There are conflicting reports in the literature as to whether saplings are more sensitive, less sensitive or of similar sensitivity to mature trees (e.g. Braun et al., 2007; Karnosky et al., 2007). In this study we use the tree response functions as a comparison to the grassland species and acknowledge that there are uncertainties in extrapolating to perennial mature trees.

In this study, only data from experiments using exposure systems close to natural conditions have been used, and results from closed chamber studies were excluded. A general concern is that the sensitivity to ozone exposure can be overestimated at the

community level due to a bias towards the use of sensitive species in fumigation experiments (Mills et al., 2007b). Although OTC experiments are designed to expose species to ozone under natural conditions, differences in microclimate between the chamber-grown plants and those growing outside may lead to differences in plant response to the same exposure concentration (Pleijel et al., 1994). In addition, this study only considered above-ground biomass responses, whereas there could have been effects on below-ground biomass for some species (e.g. Wagg et al., 2012). Also, treatment of the plants, e.g. through watering, may alter plant sensitivity to pollutants (Fuhrer et al., 1997). Furthermore, environmental conditions and inter- and intraspecific variation in response to ozone exposure make the generic applicability of the SSDs difficult (Biswas et al, 2008; Staszak et al., 2004). Some climatic factors such as high vapour pressure deficits can reduce ozone uptake through stomata. (Grunhage et al., 1997). This can lead to an overestimation of the PAF and HC_5 values related to ozone. However, high temperature and VPD conditions are comparatively rare in northern Europe and in this region climatic conditions are favorable for ozone uptake (Mills et al., 2011) and we therefore consider the concentration-based approach used in this study to be valid in this region. The current SSDs are based on a Northwest European species composition; therefore it is not possible to give an accurate prediction of the ozone effects in other regions in Europe (Paludan-Muller et al., 1999). Because of these uncertainties the geographical domain of the application of our SSDs is limited to Northwestern Europe. Flux-based ozone exposure experiments can take into account environmental conditions which are closer to observed conditions compared to the AOT40-based exposure experiments used in the current analysis (Grunhage et al., 2003; Matyssek et al. 2007). If flux models for more species become available, the SSD-concept can also be applied with stomatal flux-based exposure-response data.

The SSD concept, however, has limitations (Forbes and Forbes, 1993; Forbes et al., 2001). The relative frequency of different life-cycle types, the proportions of sensitive and insensitive taxonomic groups in communities and the role of density-dependent influences on population dynamics are not considered in the SSD concept, but are potentially important to develop sound environmental quality criteria. Competitive and facilitative interactions among plants as well as among plants and soil organisms have the potential to modify both the direction and magnitude of the O3 response (Evans & Ashmore, 1992, Hayes et al., 2010). However, some studies have clearly demonstrated that the effects of ozone in species mixtures also can be greater than those on species

grown alone or only subject to intraspecific competition (Grantz and Shrestha, 2006). A few studies have experimentally assessed the ecological significance of ozone exposure in grassland under field conditions. For example, Wedlich et al. (2012), indicate that ozone exposure in mesotrophic grassland significantly decreased the biomass of the herb fraction, however, no ozone effect was found for the grass component. They identified ozone as a dominant factor influencing species composition of the grassland community. Thwaites et al. (2006) demonstrated significant changes in species dynamics and composition in calcareous grasslands, both with positive and negative effects of ozone on different species, although total biomass and cover was not affected by ozone. Furthermore, some studies show that the species' O3 sensitivity is smaller and less frequent when plants are exposed in the field than expected from results derived from open top experiments (Bassin et al., 2007b; Stampfli & Fuhrer, 2010). On the other hand, these arguments apply as well to the SSD approach as to current critical levels, and are broad issues in all risk assessment approaches in the absence of almost any long-term community experiments in the field for grasslands.

Interpretation

The mean values of the SSDs were significantly lower for annual than for perennial grassland species. This indicates that annual grassland species, as a species assemblage, are more sensitive to ozone than perennial grassland species. This result can be explained by differences in life cycle, i.e. annual species are generally fast growing and therefore have higher stomatal flux and consequentially larger uptake of ozone (Bassin et al., 2007a; Hayes et al. 2007). Significant differences in variances were found for perennial grassland species and trees. These results can be explained by the relative small sample used to derive the SSD for trees, i.e. more species can give more variance in sensitivity. Furthermore, trees, as a species group, are more homogeneous with regard to the number of different plant families they represent (Musselman et al., 2006). However, it should also be considered that data was only available for comparatively few tree species.

The species selection, i.e. species with a biomass reduction only or all species, to determine critical ozone levels is guided by the protection objective. Conceptually, including all species in the SSD gives a more complete picture of ozone impacts on plant species communities. Statistically, however, no differences in critical levels were found between the different response types, indicating that the suggested conceptual

differences between the response types have little influence on the critical ozone levels of a species group.

HC_5 values derived in this study are lower than the equivalent critical levels recommended by the LRTAP Convention (2010). Therefore, according to the standards of conventional ecotoxicology, plant species may not be sufficiently protected with current critical levels as > 5% of species within a community may be affected at concentrations less than the current critical levels. However, the choice for the protection level of 95% of the species remains somewhat arbitrary. This may explain why the levels derived in this study are lower than current critical levels for ozone.

This study indicates that up to 20% of the species will have a 10% biomass reduction due to ambient ozone exposure. Unfortunately not enough long-term field observational studies on community level impacts of ozone exposure are available to verify the PAFs corresponding to modeled ozone concentrations (Bassin et al., 2007a; Klingberg et al., 2011). Our results of ozone impact do not fully reflect actual changes in species composition, because changes in competition and species dynamics are not taken into account. The PAF specifies the potentially affected fraction of species by ozone exposure and not the actually affected fraction.

Chapter 3

European characterization factors for damage to natural vegetation by ozone in life cycle impact assessment

Thomas M.W.J. van Goethem

Philipp Preiss

Ligia. B. Azevedo

Joachim Roos

Rainer Friedrich

Mark. A.J. Huijbregts R

Rosalie van Zelm

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Abstract

Spatially explicit characterization factors (CFs) for tropospheric ozone damage on natural vegetation caused by anthropogenic NOx and NMVOC emissions are presented for 65 European regions. The CFs were defined as the area-integrated increase in the potentially affected fraction (PAF) of trees and grassland species due to a change in emission of NOx and NMVOCs. The CF consists of a Fate Factor, quantifying the relationship between the emission of precursor substances and ozone exposure, and an area-integrated Effect Factor, quantifying the relationship between ozone exposure and the damage to natural vegetation. The relationships describing the ecological effects of a pollutant were based on a lognormal relationship between the PAF and ground level ozone concentration. We found higher CFs for NOx compared to NMVOC, and these were largest in south European regions. Furthermore, we found that both the fate factor and effect factor contribute to the spatial differences found in the CFs. Our study shows that effects caused by ozone exposure from NOx emissions are larger than those of acidification caused by NOx, indicating the importance of including ozone effects to natural vegetation in life cycle assessment studies.

Key words: Atmospheric Fate Factor; NOx and NMVOC; AOT40; trees and grassland; Effect Factor; Species Sensitivity Distribution

3.1 Introduction

Long term surveys show that tropospheric ozone background concentrations have significantly increased over recent decades, and concentrations are predicted to further increase with 0.5 – 2% per year over the next 50 years in the Northern Hemisphere (Vingarzan et al. 2004, Derwent et al 2007). Tropospheric ozone in a given area can have several sources, such as downward transport of stratospheric ozone to the troposphere or by photochemical reactions of nitrogen oxides (NOx) and non-methane volatile organic compounds (NMVOCs). NOx and NMVOC are primary precursor substances originating from anthropogenic and non-anthropogenic emissions. These pollutants can come from local sources or long-range transport (Ainsworth et al. 2012). Ozone is recognized as an important air pollutant, affecting human health and vegetation, including trees and grassland species (Ashmore 2005). Adverse effects in plants include reduction of growth and seed production, premature senescence, reduced ability to withstand stressors, and increased leaf injuries (Emberson et al. 2003).

In life cycle impact assessment (LCIA), characterization factors (CFs) estimate the environmental impact of a pollutant per unit of emission (Udo de Haes et al. 2002). Although CFs are available for human health damage caused by ozone (e.g. Van Zelm et al. 2008), studies assessing the impact to natural ecosystems have yet only included regionalized fate and exposure modeling, excluding effects on natural vegetation (Bare,. 2011, Hauschild et al. 2006, Frechette-Marleau et al. 2008). Recently, Van Goethem et al. (2013) developed quantitative exposure-effect relationships for ozone on natural vegetation (forests and natural grasslands, respectively). These relationships can be used to include ozone effects on natural ecosystems in LCIA.

The aim of this study was to determine region-specific characterization factors for damage on natural vegetation of tropospheric ozone caused by anthropogenic NOx and NMVOC emissions. The CFs were characterized for 65 European regions and subsequently compared to assess the differences in impact between the regions. Furthermore, normalization factors for ozone exposure on natural vegetation were presented. The normalization factor equals the potentially affected fraction of natural plant species in Europe due to emissions of NOx and NMVOC in 2010 per capita.

3.2 Methods

Characterization factors

The characterisation factors were defined as the area-integrated change in Potentially Affected Fraction (PAF) of forest and natural grassland species due to a change in emission of ozone precursor substances, i.e. NOx or NMVOC (in m^2.yr/kg). The CF consists of a Fate Factor (FF), quantifying the relationship between the emission of precursor substances and ozone exposure, and an Effect Factor (EF), quantifying the relationship between ozone exposure and the damage to natural vegetation. Ozone exposure is expressed as the sum of the differences between the hourly mean ozone concentration and 40 ppb during daylight hours over the relevant growing season (AOT40 in ppm.h). The CFs for ozone were calculated for 65 European regions separately as;

$$CF_{x,i,e} = \sum_j \sum_e (FF_{x,i \to j} \cdot EF_{j,e}) \quad (1)$$

where $FF_{x,i \to j}$ (ppm.h.yr/kg) is the partial fate factor representing the change in AOT40 in receiving grid j (spatial resolution of 0.5 x 0.5 degrees) following a change in the emission of substance x (i.e. NOx and NMVOCs) in region i and the effect factor $EF_{j,e}$ (m^2/ppm.h) is the change in the PAF of species of vegetation e (i.e. trees and grasslands) in grid j due to a change in ozone exposure.

Fate factor

The partial fate factor ($FF_{i \to j}$, unit: ppm.h.yr/kg) represents the change in AOT40 in a receiving compartment cell j ($\Delta AOT40_{i,j}$, unit: ppb.h) due to a change of emission of precursor x in region i (ΔM_i, [kg/yr]):

$$FF_{x,i \to j} = \frac{\Delta AOT40_j}{\Delta M_i} \quad (2)$$

The exposure is taken over time and for daytime only (Tuovinen, 2000). The AOT40 exposure index is a measure of chronic ozone exposure widely used in the risk assessment of ozone (LRTAP, 2004).

Partial fate factors for the European continent were determined with the EMEP atmospheric chemical transport model, which simulates emissions, atmospheric transport, chemical transformation, and removal from air of NOx and NMVOCs and

estimates ground level ozone concentrations (Tarrasón, 2009a). To calculate FFs for the grassland vegetation, the change in AOT40 on 1 m ground level height was used. For the trees vegetation the upper canopy height (3 m) was used. The model divides Europe into 65 emission source regions (EMEP, 2008), and receptor grid cells of 0.5°x0.5°. To derive the partial fate factors, emissions of NOx and NMVOCs are decreased by 15% compared to the baseline emission inventory for each region. The 15% represents a realistic "quasi-marginal" change of emissions but still allows to assume sufficient linearity and to downscale the change of impacts to a unit of emission change (Tarrasón 2009b). FFs were determined for each region, precursor pollutant, and 2010 background emissions. The emission data set for 2010 corresponds to the baseline Current Legislation (CLE) scenario, developed by IIASA for the development of the Thematic Strategy on Air (Amann et al., 2008; Tarrasón, 2009b). Because of inter-annual variability in the meteorology, average results based on meteorological years 1996, 1997, 1998, and 2000 were derived as these years represent typical conditions (Tarrasón, 2009a).

Effect factor

EFs were derived via the following steps. First, species-specific AOT40 exposure-biomass response functions, as reported by Van Goethem et al. (2013), were used to derive EC50 values for trees and grassland species. The species-specific EC50 equals the AOT40 at which there is a 50% reduction in biomass compared to a situation with no ozone over-exposure, i.e. AOT40 = 0. We selected the EC50, as it follows the same approach employed for toxicity in LCA (see e.g. Rosenbaum et al., 2008). Note that some species showed to be insensitive to ozone exposure, i.e. no EC50 value was derived for these species. In a second step, we used the EC50-values to derive a Species Sensitivity Distribution (SSD) for respectively forest and natural grassland species, taking into account the fraction of species with no biomass decrease. An SSDs represents a cumulative stressor-response distribution based on single-species sensitivity data. Assuming a lognormal species sensitivity distribution for ozone exposure, the PAF can be derived as:

$$PAF_{j,e} = \frac{1 - f_{nbd}}{\sigma_e \cdot \sqrt{2 \cdot \pi} \cdot AOT40_{j,e} \cdot \ln 10} \cdot \int_0^{AOT40} \exp\left(-\frac{1}{2} \cdot \left(\frac{\log(AOT40_{j,e}) - \mu_e}{\sigma_e} \right)^2 \right) dAOT40 \quad (3)$$

where $AOT40_{j,e}$ represents the ambient ozone concentration in grid j of vegetation type e (either forest or natural grassland), μ_e is the average of the ^{10}logEC50 values for ozone

in AOT40-units (ppm.h), as observed for different species in vegetation type e, f_{nbd} is the fraction of species with no biomass decrease and σ_e is the standard deviation of the ^{10}logEC50-data within vegetation type e.

In a third step, we calculated the marginal change in PAF due to the marginal change in ground level ozone exposure (in ppm.h), equal to the derivative of equation 3, via:

$$\frac{\partial PAF_{j,e}}{\partial AOT40_j} = \frac{1 - f_{nbd}}{\sigma_e \cdot \sqrt{2 \cdot \pi} \cdot AOT40_{j,e} \cdot \ln 10} \cdot \exp\left(-\frac{1}{2} \cdot \left(\frac{\log(AOT40_{j,e}) - \mu_e}{\sigma_e} \right)^2 \right) \quad (4)$$

In a final step. the grid-specific marginal effect factor (MEF) per vegetation type was defined as:

$$MEF_{j,e} = \frac{\partial PAF_{j,e}}{\partial AOT40_j} \cdot A_{j,e} \quad (5)$$

, where $A_{j,e}$ is the area (m^2) occupied by vegetation type e in grid j.

The $AOT40_{j,e}$ data were based on grid-specific background AOT40 concentrations for 2010 determined by the EMEP model (Simpson et al., 2003). For the grassland vegetation, AOT40 values based on a growing season of May-July and a ground level height of 1m were used. For the trees vegetation, April-September and the upper canopy height (3 m) was used. The Global Land Cover 2000 (GLC2000) database was used to calculate the grid-specific area occupied by each vegetation type (Bartholomé and Belward, 2005). Classification of GLC2000 types into trees and grasslands can be found in table S1.

Sensitivity analysis

To test the sensitivity of the CF regarding the relationship describing the ecological effects of ozone on natural vegetation, the marginal effect factor (MEF) was compared to two other options to calculate an effect factor. This was done because there is no consensus yet in the best way to derive an EF (Huijbregts et al., 2011).

A simplified EF, assuming a linear change in PAF with changing AOT40 represents the average effect between a PAF of 0.5 and 0. This linear method is commonly used in ecotoxicology (Pennington et al. 2004) :

$$LEF_{j,e} = \frac{\Delta PAF_{j,e}}{\Delta AOT40_j} \cdot A_{j,e} = \frac{0.5 \cdot (1 - f_{nbd})}{10^{\mu}} \cdot A_{j,e} \quad (6)$$

, where 10^{μ} is the AOT40 value that affects 50% of the species in vegetation type e in grid *j*.

For the average effect factor (AEF), it is assumed that the distance between the current and desired situation is proportionally distributed over the stressor range. And, as opposed to LEF, the actual concentration of AOT40 in grid j ($AOT40_j$) estimates the average distance to the PAF at 0 (Huijbregts et al., 2011). The average distance between the current state and the preferred state of the environment can be calculated as:

$$AEF_{j,e} = \frac{\Delta PAF_{j,e}}{\Delta AOT40_j} \cdot A_{j,e} = \frac{PAF_{j,e}}{AOT40_j} \cdot A_{j,e} \quad (7)$$

, where $PAF_{j,e}$ is related to the $AOT40_{j,e}$ representing the ambient ozone concentration in grid j of vegetation type e.

Normalization factor

The normalization factors (NF) for ozone impacts in Europe were derived by multiplication of the region-specific characterization factors with the substance-specific emissions in each region of 2010, divided by the total population in all regions (Table S3.2) (Verstreng et al., 2012). The normalization factor equals the area-integrated potentially affected fraction of natural plant species in Europe due to emissions in 2010 of NOx and NMVOC per capita (in PAF.m^2/capita):

$$NF = \frac{\sum_x \sum_i (M_{x,i} \cdot CF_{x,i})}{\sum_i (N_{pop,i})} \quad (8)$$

, where NF is the normalization factor of the summation of all 65 regions. $M_{x,i}$ is the emission of precursor x (NMVOC or NOx) in region i (in kg/yr), $CF_{x,i}$ is the characterization factor for substance x in region i and $N_{pop,i}$ is the number of inhabitants in region i (CIESIN, 2005).

Statistical analysis

Pearson correlation test was used to determine linear correlation between CF and total Fate Factor to have an indication if the FF contributes most to the variation in CF results for emissions of both NMVOC and NOx.

3.3 Results

Species Sensitivity Distributions

Figure 3.1 shows the species sensitivity distributions for grassland species and trees based on EC_{50}-data with the fraction of species with no biomass decrease included. The SSDs were based on 87 grassland species and 9 tree species. The percentage of species in the dataset that exhibited a biomass reduction was 71% for grassland species and 100% for tree species.

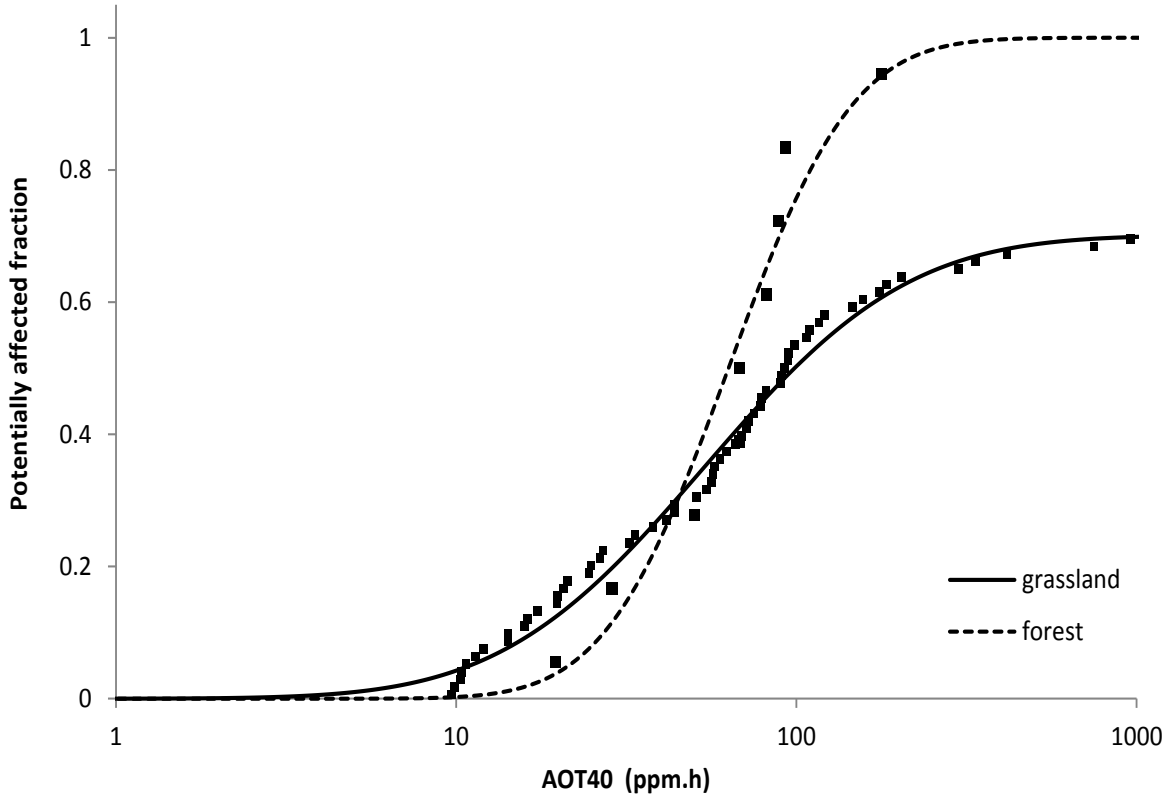

Figure 3.1. Species sensitivity distributions based on EC50 values for trees (n=9) and grassland (n=87) using a lognormal distribution.

Characterization factors

Characterization factors for damage to natural vegetation by tropospheric ozone were calculated for 65 European regions both for NMVOC and NOx (in m^2.yr/kg) (Fig. 3.2). The full region-specific results are given in the SI (Table S3.3). The region-specific CFs and emission weighted CFs for Europe are included in table S3.3. Weighing was done based on region specific emissions in 2010 (table S3.2) (Verstreng et al., 2012). The CFs for ozone damage due to NMVOC emissions range from 0.3 to 5.0 m^2.yr/kg, with smallest CFs for Sweden and Finland and largest for Luxembourg and Italy. The CFs for NOx emissions range from -0.3 to 20.6 m^2.yr/kg, with smallest a negative CF for the Netherlands and largest CFs for France and Switzerland. The negative CFs for NOx indicate that increased emissions will actually lead to a net reduced ozone exposure. Overall, the CFs for NOx emissions are largest in South European regions. For NMVOC emissions CFs are largest for regions in Central Europe.

The correlation between CF and the total FF was tested . This was done by plotting the characterization factors versus the sum of the partial fate factors (∑FF) for every emission region (Fig. 3.3). A correlation between CF and ∑FF was found by using the Pearson correlation test, r-values of 0.73 and 0.70 were found for NMVOC and NOx respectively. Both r-values were reported with a p-value <0.0001. However, the R-squares of the linear regressions, 0.53 and 0.49 for NMVOC and NOx, imply that both the fate and effect factors contribute significantly to the spatial differences found in the CF.

Variability in EF is determined by variability in (1) the grid-specific area covered by each vegetation type and (2) grid-specific AOT40 values (see fig. 3.4). The area covered by grassland and forest varies across Europe (fig. S3.1). Therefore, the relative contribution of the grassland and forest types to the EF varies by grid (fig. S3.2). Variability in FF is also determined by the grid-specific area covered by each vegetation type, because the change in ozone exposure is determined for a different height per vegetation type (fig. S3.1, S3.2).

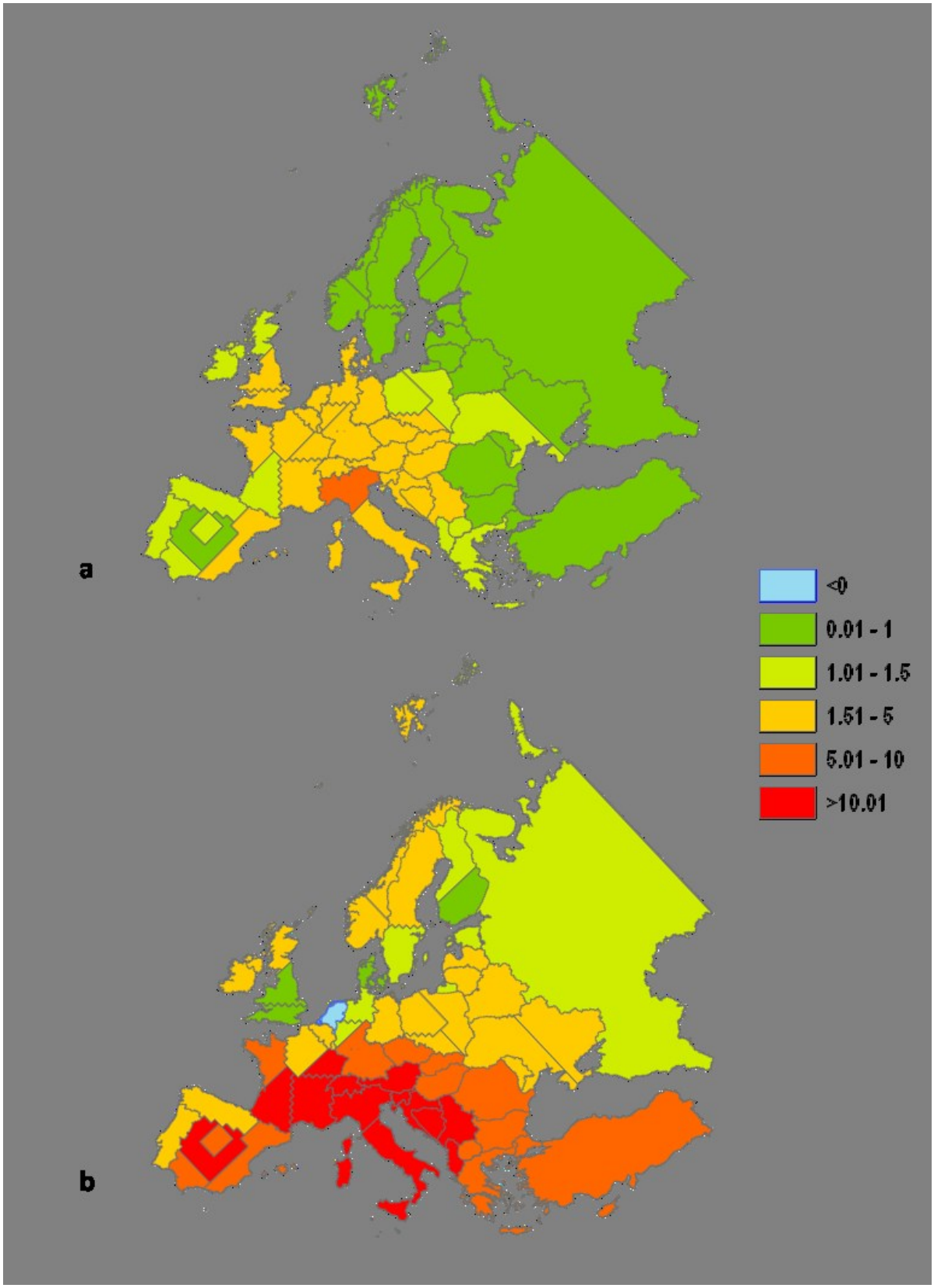

Figure 3.2. Region specific characterization factors for damage due to ozone caused by emissions of (a) NMVOC and (b) NOx (in m^2.yr/kg).

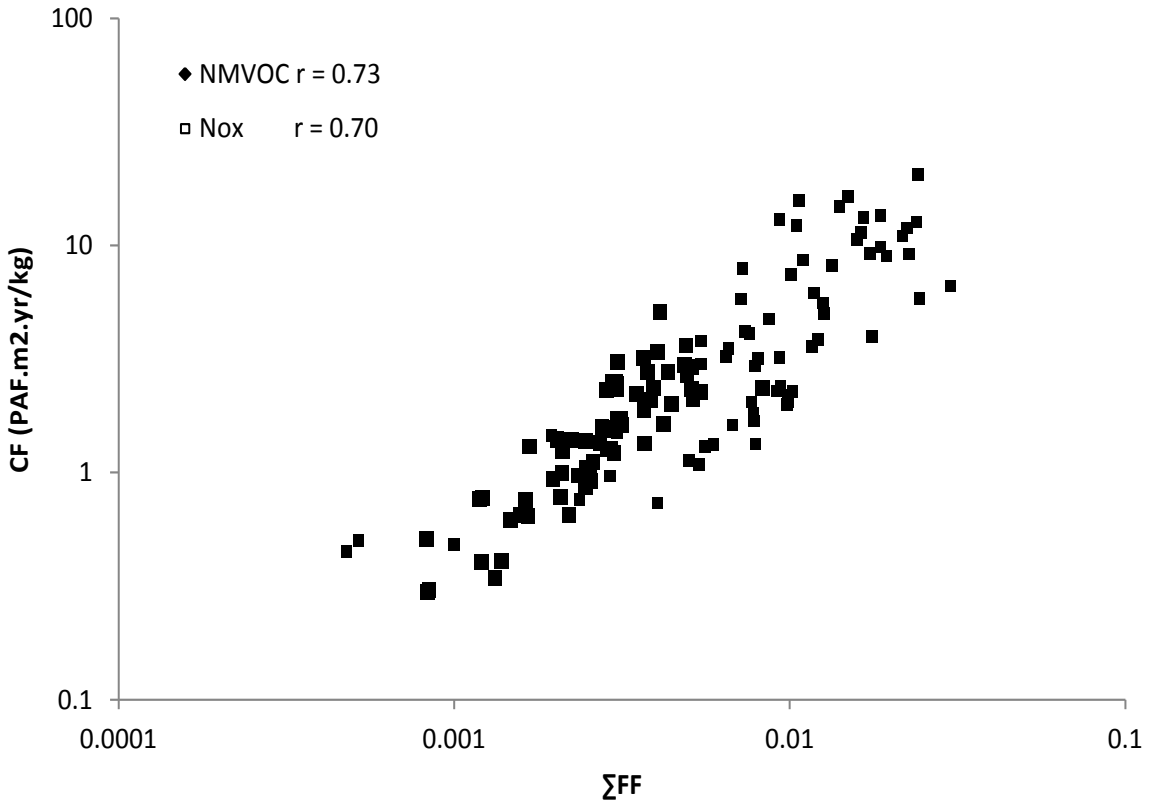

Figure 3.3. Correlation between total fate factors (i.e sum of grid specific partial Fate Factor (FF) of each region, in ppm.h.yr/kg) and Characterization Factor (CF) for NMVOC and NOx. Results of the pearson correlation are shown in the top left of the graph.

Normalization factor

The normalization factor for ozone impact on natural vegetation due to emissions of NOx and NMVOC in 2010 is $1.4 \cdot 10^{-10}$ m^2 / capita. NOx contributed 80% and NMVOCs 20% to the normalization factor, implying that NOx is the main contributor to damage by ozone exposure in natural vegetation in Europe.

Sensitivity analysis

The different EF types for both forests and natural grasslands are plotted against the grid-specific background AOT40 values in Figure 3.4, along with the frequency of AOT40 background exposure values in Europe. Most AOT40 exposure values range from 0.01 to 1 ppm.h. In this range the LEF is generally 2 orders of magnitude larger than both the MEF and AEF. Here, AEF is also larger than the MEF. In higher AOT40 ranges, 10 to 100 ppm.h, the MEF is larger than the LEF and AEF.

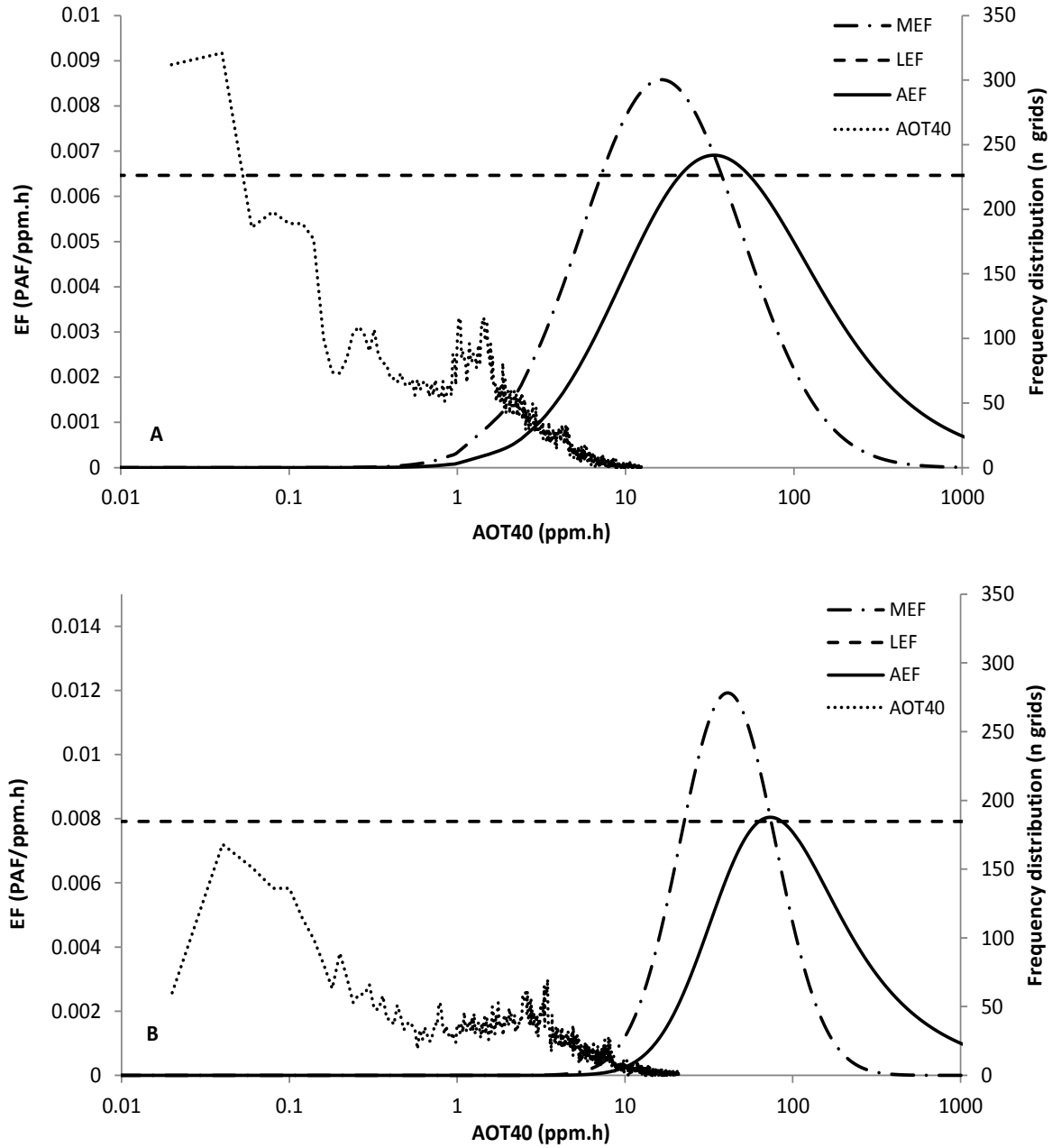

Figure 3.4. Marginal Effect Factors (MEF), Linear Effect Factors (LEF) and Average Effect Factors (AEF) (with area excluded) for (a) grasslands and (b) forests (left vertical axis). And AOT40 values per grid (right vertical axis) for grasslands and forest plotted as a frequency distribution with steps of 0.02 ppm.h.

The linear effect factor method results, on average, in CFs that are a factor of 3 larger than the CFs calculated with the marginal effect method. On the other hand, the average effect factor method results in CFs that are typically a factor of 7 smaller compared to CFs calculated with the marginal effect method. Correlation between the CFs based on the marginal approach versus the CFs based on the linear and average

approach was large (Pearson correlation test, r^2-values of 0.81 and 0.89, respectively. See Figure S3.3).

3.4 Discussion

In this study, characterization factors representing damage to natural vegetation by ozone exposure were calculated for unit emissions of NMVOC and NOx in 65 European regions. Furthermore, normalization factors were calculated, which showed the potentially affected fraction of plant species over a certain area due to European emissions of NMVOC and NOx per capita. In the following, we discuss the benefits and limitations of the calculation procedure and provide an interpretation of the results obtained. Furthermore, our work is compared to another impact category, i.e. damage due to ozone is compared to damage due to acidification.

Uncertainties

Fate factor

The Unified Eulerian EMEP model has been selected to derive fate factors for ozone exposure. Validation studies of the EMEP model show that EMEP gives a description of ozone formation in Europe that is in good agreement with the much more comprehensive IVL chemistry model and observational data (Andersson-Sköld and Simpson, 1999, Hov et al. 1978, Simpson 1992). Furthermore, intercomparison studies of atmospheric chemistry-transport models, comparing EMEP with, e.g. the LOTOS-EUROS and TM5 model, indicate that the EMEP model gives results, which are in line with measured summer daytime averages, maxima and the diurnal cycle, which are important for modeling AOT40 (Vautard et al., 2007; Van Loon et al., 2007).

Uncertainty in the derivation of fate factors for ozone formation relates to the complex non-linear chemistry of photochemical ozone creation, including the interaction between the precursors NOx and NMVOC, and meteorological conditions (Simpson et al., 2003; Solberg et al. 2004). Emission inventories are a large source of uncertainty in model predictions (Atkinson, 2000). Meteorologically induced variability of AOT40 shows a gradient decreasing from north-west to south-east Europe (EEA, 2009). The variability was estimated as approximately 10% for southern Europe, 20–30% in central Europe and 50% or more in the United Kingdom (EEA, 2009). Further uncertainty arises due to the fact that different NMVOC substances have different potential to create ozone

(Andersson-Sköld and Holmberg 2000). The mixture of different NMVOCs can differ across regions and sectors. However, the current FF is an average for the common mix of NMVOCs. The model can be improved by including sector dependent NMVOC speciation or even substance-specific model runs (Derwent et al., 2007).

Because only the EMEP regions are covered, receptor areas outside this area are not taken into account in the fate factor calculations. As a result, the area-integrated ozone exposure will be underestimated for NMVOC and NOx emissions, because impacts due to ozone outside of the receptor area are not taken into account.

Effect factor

The GLC2000 database was used to calculate the grid-specific area occupied by each vegetation type. However, not all GLC2000 land cover classes corresponded in terms of species composition to our classification in vegetation types, therefore making the right allocation for some of the classes uncertain, especially for transitional vegetation (Table S3.1). For instance, the shrub cover class was appointed to the grassland type but it might contain tree species as well. Making a more detailed division in vegetation types, however, was not possible because of lack of detailed response data for a wide range of taxonomic groups.

The relationships between ozone exposure and damage for forests and grasslands were calculated using species-specific exposure-response data based on experimental studies (Van Goethem et al., 2013). However, in contrast to Van Goethem et al. (2013) no distinction was made between annual and perennial grassland species, because no grid-specific data on the area each species group occupied was available. A general concern regarding the exposure-response data is that the sensitivity to ozone exposure can be overestimated at the community level due to a bias towards the use of sensitive species in fumigation experiments, therefore leading to overestimated CFs (Mills et al., 2007). Furthermore, there are uncertainties regarding variation in atmospheric conditions and species responses (Fuhrer, 2002). In literature significant inter- and intraspecific variation in response to ozone exposure was reported for species occurring in multiple regions (Oksanen et al., 2001). This variation is mostly explained by differences in climate, for instance, climatic factors such as high vapour pressure deficits can reduce ozone uptake through stomata (Biswas et al, 2008). This implies that region-specific SSDs are needed to show the distinctly different environmental conditions and species assemblages, e.g. on an ecoregion or biome level. This was, however, not possible due

to lack of available data. (Van Goethem et al., 2013). Furthermore, there is larger uncertainty in the tree SSD because it was based on 9 tree species compared to 61 species for grassland. However, the EF for grassland has a higher contribution to the CF compared to the EF for forests, therefore limiting overall uncertainty (fig. S3.4). The receptor grid resolution and sizes also influence the EF. For example, the EF results get more accurate at higher resolution when the vegetation is heterogeneously distributed in the grids. Besides the AOT40 approach, which is based on ozone concentrations only, another index is currently in use for indicating risk of ozone damage to natural vegetation in Europe. This approach, i.e. the stomatal ozone flux or Phyto-toxic Ozone Dose (POD), involves estimating the amount of ozone entering via the stomata of vegetation, which, on biological grounds, would yield more elaborate response models (Mills et al., 2011). Comparison between these approaches indicates a different ozone exposure distribution across Europe (Simpson et al., 2007). Currently, however, there are not enough species-specific flux-based exposure-effect models available to derive EFs and subsequent CFs based on the POD approach.

The marginal approach to calculate effect factors was used as the default in our study, the interest in LCIA generally lies in assessing the influence of small emission changes (Van Zelm et al., 2009; Struijs et al., 2011). However, alternative approaches found in the literature are linear effect factors (LEFs) and average effect factors (AEFs) (Huijbregts et al., 2011). The linear approach is commonly used in other impact categories, such as toxicity, in case no reliable background pollutant concentrations are available (Rosenbaum et al., 2008). As the linear approach disregards the shape of the exposure-response curve, it is considered to be less reliable compared to the marginal or the average approach. The AEF represents the average distance between the current and the preferred state of the environment per unit of emission (Huijbregts et al., 2011). The virtue of the AEF approach is that it focuses on the total change of ozone exposure that is required to reach the preferred state of the environment. The correlations found between the CFs based on the different effect factor methods imply that they largely result in the same ranking between regions and substances (fig. S3.3). Looking at the absolute CF values, the choice for either the marginal or average effect factor method gives a typical difference in the CFs of a factor 7. This implies that the choice for a marginal or an average approach has a substantial influence on the CFs. Therefore, when comparing to other impact categories, e.g. toxic impacts, for which the approach is different the choice for an effect factor model is important.

Interpretation of the results

Tropospheric ozone formation is driven by complex non-linear photochemistry between the precursor substances NMVOC, NO_x and background tropospheric ozone (Atkinson, 2000; Butkovic et al., 1990). For instance, at nighttime and in the immediate vicinity of very large emissions of NO, ozone concentrations are depressed through the process of NO_x titration (Sillman, 2003). This consists of the removal of ozone through reaction with NO. Generally, summer ozone levels in Europe show an increasing gradient from the north-west to the south-east part. In the Mediterranean, large scale circulation cells are established and coastal emissions can be trapped for several days in land-sea breeze circulation systems (Borrego et al., 1995). These effects in relation with larger solar radiation levels in southern Europe can result in high ozone levels. This is also reflected in our results, were characterization factors for NOx are largest in south European regions, which are the result of larger AOT40 levels per unit of emission (FF) and consequently also higher EFs. The CF for NOx is on average 3.5 times larger than the CF for NMVOC. These findings correspond with the results of Hauschild et al. (2006), who found larger CFs for NOx by a factor of 3.6. The negative characterization factor in the Netherlands for NOx indicates that increased emissions will actually lead to reduced ozone formation. This is caused by the titration effect (Simpson et al., 2003). The normalization factor for ozone impact also indicates that NOx is the main cause of ozone damage to natural vegetation. This difference is caused by both larger CFs and larger emissions for NOx, i.e. the emissions for NOx are on average 1.5 times larger compared to those for NMVOC.

The ILCD handbook recommends to provide CFs for carbon monoxide and methane as well (EC-JRC, 2011). However, the importance of carbon monoxide and methane in ozone formation compared to NMVOC and NOx is much lower (Wayne, 2000), hence the potential contribution of these substances to tropospheric ozone formation was not addressed in this paper.

Our results for NOx can be compared with those for acidification by Van Zelm et al. (2007) and Roy et al. (2013). The CF for NOx related to acidification of European forests of Van Zelm et al. (2007) was 0.37 m^2·yr/kg for a time horizon of 500 years. Our emission weighted CF for ozone damage to forests by NOx emissions in Europe was 3.4 m^2·yr/kg. Roy et al. (2013) reported a European acidification CF for NOx of 5.0 m^2·yr/kg, while our CF for ozone damage of NOx is 5.6 m^2·yr/kg. These results indicate a larger effect due to

ozone damage by NOx emissions than that of acidification by NOx emissions, showing the importance of including ozone damage to natural vegetation in LCIA. This result is confirmed in literature as the contribution of NO_x emissions to ground-level ozone formation, acidification, and eutrophication is considered of comparable importance (Johansson et al., 2001). Furthermore, ozone is also identified as an important pollutant with regards to ecosystem damage when compared to acidification (Ashmore, 2005).

Summarizing, we determined spatially explicit characterization factors for damage of tropospheric ozone by anthropogenic NOx and NMVOC emissions on natural vegetation in 65 European regions. It is the first time that CFs for ozone were derived for impacts on natural vegetation on an endpoint level. Fate factors were derived with the EMEP atmospheric chemical transport model. The effect factors were based on a lognormal relationship between the potentially affected fraction of species and ground level AOT40. We found that NOx is the major contributor to damage caused by ozone. Furthermore, we found that both the fate and effect factors contribute to the spatial differences found in the CFs. Our study shows that effects caused by ozone exposure from NOx emissions is larger than that of acidification caused by NOx.

Chapter 4

How to assess species richness along single environmental gradients? Implications of potential versus realized species distributions

Thomas M.W. J. van Goethem

Mark A.J. Huijbregts

Wieger G.W. Wamelink

Aafke M. Schipper

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Abstract

Quantifying relationships between species richness and single environmental factors is challenging as species richness typically depends on multiple environmental factors. Recently, various methods have been proposed to tackle this challenge. Using a dataset comprising field observations of grassland vegetation and measured pH values, we compared three methods for deriving species richness response curves. One of the methods estimates species richness close to the maximum species richness observed at the sites, whereas the other two provide estimates of the potential species richness along the environmental gradient. Our response curves suggest that potential species richness of grasslands is slightly more sensitive to acidification than realized plant species richness. However, differences in corresponding environmental quality standards (EQS) for acidification were small compared to intrinsic spatial differences in natural soil pH, indicating that natural background values are more important to consider in the derivation of EQS for pH than methodological differences between the three approaches.

Key words: Survey data; pH; Stressor-Response Relationships; Species Sensitivity Distributions (SSDs); Environmental Quality Standards (EQS)

4.1 Introduction

Environmental factors that determine species distribution patterns and species richness are of primary interest to nature conservation (Pausas & Austin, 2001). Quantifying the influence of individual factors on species communities in a systematic way can help to improve our understanding and predictive ability of biodiversity patterns, derive environmental quality standards, and underpin abatement priorities (Latour and Reiling, 1993; Latour et al., 1994; Van Goethem et al., 2013; Wamelink et al., 2013). However, species distributions are typically dependent on multiple environmental factors, including both abiotic and biotic drivers (Pulliam, 2000; Schipper et al., 2014; Soberón, 2007). As confounding environmental factors generally result in considerable scatter among species richness observations, it is not straightforward to extract relationships between species richness and single factors from field data (Cade and Noon, 2003; Van den Brink et al., 2002).

Recently, various methods have been proposed to tackle this challenge (Leung et al., 2005; Struijs et al., 2011; Kefford et al., 2011; Iwasaki & Ormerod, 2012; Azevedo et al., 2013; Cormier & Suter II, 2013). Most of these methods are based on occurrence data (e.g. presence-absence data), which are generally more readily available than abundance data (Pearce & Boyce, 2006; Potts & Elith, 2006). One method is to relate site-specific observations of the number of species present to a particular environmental variable with quantile regression (Iwasaki & Ormerod, 2012). Most regression techniques relate changes in the mean of a response variable to one or more explanatory variables. With quantile regression, any part of the distribution of a variable can be used as response (Cade and Noon 2003). Quantile regression based on one of the upper boundaries of the response variable distribution (e.g. the 0.95 or 0.99 quantile) is expected to show the constraints imposed by the explanatory environmental variable of concern (Iwasaki & Ormerod, 2012; Lancaster and Belyea, 2006). A second method is to assess the number of species present within regular intervals along a particular environmental gradient by pooling multiple samples per interval (‘pooled samples method’). The number of species per interval is then assessed either by simply counting the number of unique species across all samples within the interval (Struijs et al., 2011) or by establishing a species accumulation curve (SAC) per interval, thus correcting for potential differences in the number of samples between the intervals (Kefford et al., 2011). With a third method, observations of multiple species across multiple samples are used to first establish species-specific occurrence ranges, represented by the

minimum and maximum values of the environmental variable of concern where the species has been observed. These occurrence ranges are then stacked across the species to arrive at an estimate of species richness ('occurrence range method'; Verbrugge et al., 2012, Azevedo et al., 2013, Cormier et al., 2013b).

Given the differences in approach, these three methods are expected to yield different species richness estimates, reflecting differences in potential and realized species richness. Potential species richness refers to the species that could occur at a specific site, while realized species richness refers to the species that actually occur there (Jiménez-Valverde et al., 2008). By modelling an upper quantile of the distribution of species richness actually observed at the sampling sites, the quantile regression method yields an estimate of the maximum species richness that may be realized at a particular location with a given pH. In contrast, the other two methods yield species richness estimates representing the pool of plant species corresponding with a given pH, i.e., the potential species richness. Species richness typically increases with an increasing number of samples (Kefford et al., 2011). Hence, aggregating observations from multiple sampling sites at each given interval along a particular environmental gradient, as is done in the pooled samples method, is expected to yield considerably higher values of species richness than can be observed at specific sampling sites (Kefford et al., 2011). The occurrence range method, finally, is expected to yield the highest estimates of species richness, by aggregating the species occurrences over the full environmental gradient rather than for each given interval separately.

The goal of this paper was to compare the three methods by applying them to the same species-environment dataset and quantifying the differences in the resulting species richness response curves. The dataset comprises presence-absence observations of terrestrial plant species along a gradient of soil pH measurements (pH 3-10) collected from 4412 sampling sites of grassland vegetation across the Netherlands (Wamelink et al., 2012). The methods were compared by quantifying the shapes of the response curves (magnitude, width) along the pH gradient. Furthermore, we compared the methods in terms of environmental quality standards, i.e. the pH levels corresponding with a predefined relative reduction in species richness (Van Straalen & Denneman, 1989; Posthuma et al., 2002). To achieve this we converted the species richness estimates to relative values with a maximum of 100%, thus obtaining field-based species sensitivity distributions (f-SSDs), i.e., empirical distributions describing interspecies variation in sensitivity to a particular environmental variable.

4.2 Methods

Species richness response curves

Quantile regression

The quantile regression method to estimate species richness along the pH gradient was based on Cade & Noon (2003). In our study, three models were constructed at the 95% quantile (Visser & Sasser, 2009): a linear model ($y = \beta_0 + \beta_1 \cdot x$), a Gaussian model ($y = \beta_0 + \beta_1 \cdot x + \beta_2 \cdot x^2$) and a baseline model where species richness is estimated by a constant (i.e., an intercept-only model). The most parsimonious model was selected based on the Bayesian Information Criterion (Lee et al., 2013). The different models were also constructed for the 97.5% and 99% quantiles to assess the influence of the quantile selection on the species richness estimates. The quantile regression was performed with the quantreg package in R (Koenkers et al., 2013).

Pooled samples method

With the pooled samples method (Kefford et al., 2011), we derived species accumulation curves (SACs) for each interval i along the pH gradient. The SACs were derived using a resampling rarefaction method (100 times) that calculates the mean number of species observed (SR_{est}) in 1 to n samples, where n is the total number of samples pooled. The SR_{est} in k samples, SR_{est} (k), is the mean number of species estimated in k samples. The SR_{est} (inf) is the mean number of species where one added sample leads to a maximum increase of less than one species (Verberk et al., 2006). For each interval i we considered the SR_{est} (inf) as an estimate for $SR_{i,j}$ (Kefford et al., 2011). The intervals i were set at 0.1 pH unit, so that there were enough observations in each interval to derive SR_{est} (inf) (Table S2). The SACs were extrapolated up to a maximum of 5 times to ensure that SR_{est} (inf) could be estimated for all intervals (Colwell, 2012). As a sensitivity check the response curves were also derived based on 50, 20 and 1 samples. The SACs were determined using the computer software EstimateS 7.5.1 (Colwell, 2004).

Occurrence range method

Following (Azevedo et al. 2013a), we defined the occurrence range for each species as the range between minimum and maximum pH values corresponding to the occurrence of that species as observed in the field. A species was considered to be absent at pH values outside this range, and potentially present at values inside its occurrence range.

Species richness (SR_i) was computed as the number of species potentially present at each pH interval i as

$$SR_i = \sum_s O_{s,i} \qquad \text{(Eq. 1)}$$

where $O_{s,I}$ is the occurrence of each species s at pH interval i, with O = 0 when the pH value is outside a species' occurrence range and O = 1 if the pH value is within its occurrence range. The intervals i for pH were set at 0.1. To assess the sensitivity of SR to changes in occurrence ranges, the species occurrences were also derived based on the 5^{th} and 95^{th} and 2.5^{th} and 97.5^{th} percentiles of the pH values corresponding to the field occurrence of that species.

Dataset

The ecological conditions (EC) database compiled by Wamelink et al. (2012) was used in this study. This database comprised vegetation relevés from the Netherlands, each accompanied by a measured value of at least one abiotic soil parameter. The database contained 5243 grassland relevés with a measured pH value, covering the period from 1936 to 2011 (Table 4.1). pH values were measured in H_2O extract and ranged from 3.0 to 10.1. Several relevés were part of a time series: the dataset included 141 sites where a relevé was made at least twice. To remove potential confounding influences of temporal autocorrelation, we included only the most recently recorded relevés from each time series in the dataset. This led to a decrease in the number of releves of 5243 to 4412. The vegetation relevés were made according to the Braun-Blanquet method and followed the syntaxonomical classification of Schaminée et al. (1995)(Braun-Blanquet, 1921). In total 1321 species were recorded in the relevés. More details regarding the EC database can be found in Wamelink et al. (2012).

Table 4.1. Characteristics (Mean, SD, Median, Min, Max and various percentiles) of the measured pH values and species richness for 4412 relevés.

	Mean	SD	Median	Min	0.025	0.25	0.75	0.975	Max
pH values	5.7	1.3	5.6	3.0	3.8	4.7	6.4	7.9	10.1
Species richness	25	12	24	1	5	14	31	48	73

Estimated vs. observed species richness

We compared the estimated species richness (SR_{est}) with the observed species richness (SR_{obs}) over the relevés by deriving the average relative difference over the pH gradient as

$$RD_{est-obs} = \frac{1}{N_i} \sum_i \frac{SR_{est,i} - \overline{SR_{obs,i}}}{\overline{SR_{obs,i}}} \qquad \text{(Eq. 2)}$$

where $SR_{,est,j}$ represents the species richness estimated for interval i and N_j is the total number of intervals.

Field-based species sensitivity distributions (f-SSDs) and environmental quality standards (EQS)

We derived a field-based species sensitivity distribution (f-SSD), an approach developed in the field of ecotoxicology, from each of the three species response curves. To that end, the estimated species richness was transformed into a zero-to-one measure, the relative species richness (r-SR), as

$$rSR_i = \frac{SR_i}{SR_{\max}} \qquad \text{(Eq. 3)}$$

where SR_{max} for a given method represents the highest species richness estimated in any interval i along the pH gradient. The maximum r-SR (i.e., r-SR = 1) is obtained if the species richness in a particular interval i equals SR_{max}, while r-SR = 0 represents the complete absence of species. The resulting f-SSDs thus represent changes in species

richness in relation to the pH gradient, relative to the highest species richness estimated for a particular pH level in the study area. Subsequently, for the pooled samples and occurrence range methods, we applied least squares regression to the r-SR estimates to obtain an explicit function of r-SR in relation to pH. Next, we used the resulting functions to derive environmental quality standards (EQS). We defined the EQS as the pH value corresponding with a 5% reduction of the species richness due to acidification (Posthuma et al., 2002).

4.3 Results

Species richness response curves

The response curves based on the quantile regression, pooled samples and occurrence range methods all showed a unimodal response along the pH gradient (pH 3-10) (Figure 4.1). Optimum pH values were found in the range of 6.1-6.5 (Table 4.2). The response curves differed in their width and relative amplitude, where width is defined as the pH range at half SR_{max} and relative amplitude as the relative difference between maximum and minimum species richness estimated along the pH gradient (Table 4.2). The widths ranged from 4.7 units for the occurrence range method to 6.9 units for the quantile regression method. The relative amplitude ranged from 0.56 for the quantile regression method to 1.0 for the occurrence range method.

Table 4.2. Optimum pH (pH_{max}), the width at 0.5 SR (width $SR_{0.5}$) and relative amplitude of the species richness response curves, maximum SR (SR_{max}), average relative difference between the estimated SR and the observed SR ($RD_{est\text{-}obs}$) for each of the response curve methods.

	Quantile Regression	Pooled Samples	Occurrence Range
pH_{max}*	6.5	6.1	6.3
Width $SR_{0.5}$*	6.9	5.1	4.7
Relative amplitude*	0.56	0.76	1.00
SR_{max}	50	590	702
$RD_{est\text{-}obs}$	1.1	16.0	17.6

*pH_{max}, width $SR_{0.5}$ and relative amplitude based on the f-SSDs

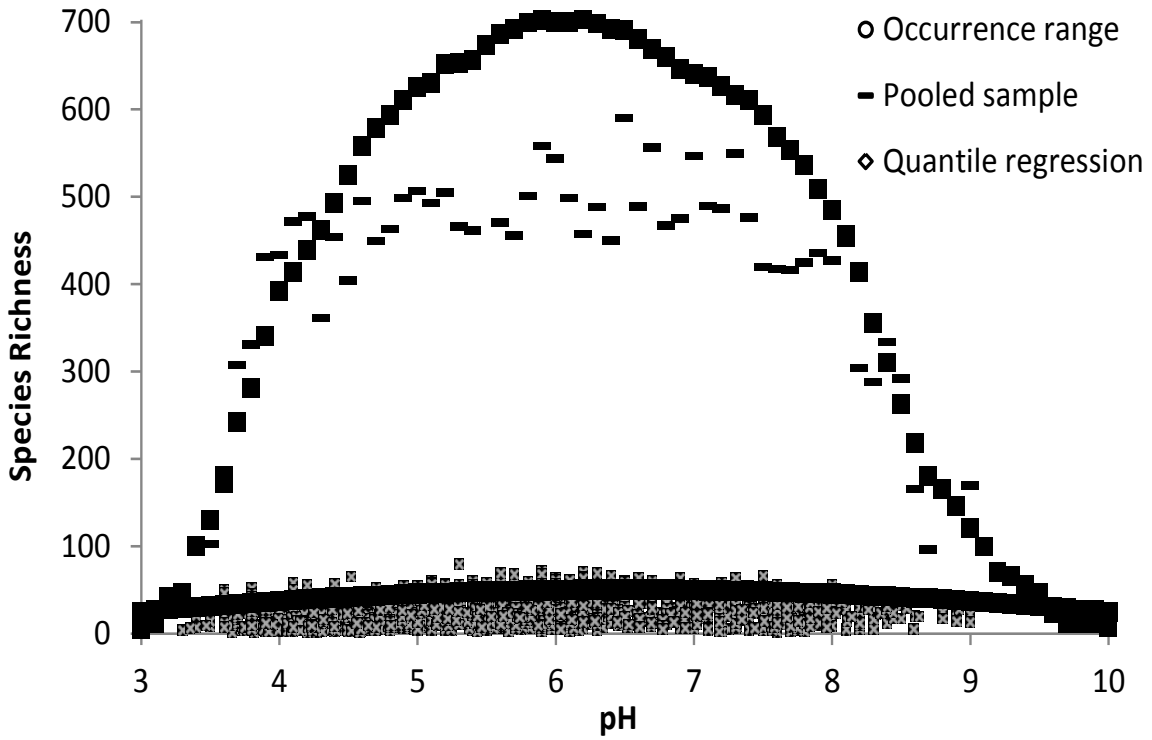

Figure 4.1. Field-based species richness response curves for pH derived with the quantile regression method, pooled samples method and the occurrence range method. Observed SR is plotted in gray. In the quantile regression method the Gaussian model was selected as the most parsimonious model based on the 0.95 quantile (Table S1; Figure S1). Confidence intervals for the SR estimates derived with the pooled sample method can be found in Figure S2.

Comparison with observed species richness

The maximum estimated species richness was 50 for the quantile regression method, 590 for the pooled sample method and 702 for the occurrence range method (Table 4.2). The response curve based on the quantile regression method followed the highest observed species richness in the field, whereas the pooled samples and occurrence range methods estimated much higher SR (Figure 4.1). The average relative difference between SR_{est} and SR_{obs} ranged from 1.1 for the quantile regression method to 17.6 for the occurrence range method (Table 4.2). Per method, the average observed SR and estimated SR per interval are given in Table S4.2. Based on the average relative difference between the estimated and observed SR, the quantile regression method is placed on the left of the gradient from realized to potential species richness, whereas the occurrence range and pooled samples methods are placed towards the right (Figure 4.2).

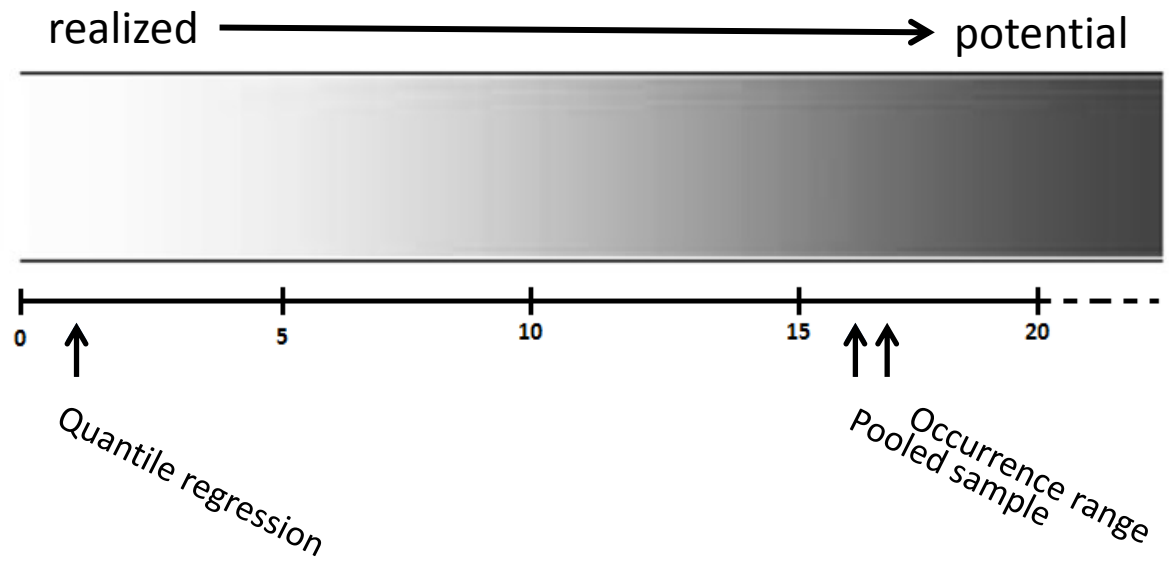

Figure 4.2. Representation of the three response curve methods on a gradient from realized to potential species richness (adapted from Jiménez-Valverde et al., 2008). Numbers on the axis indicate the average relative difference between the estimated and average observed SR for each of the response curve methods.

Field-based species sensitivity distributions and environmental quality standards

Field-based species sensitivity distribution (f-SSDs) for the quantile regression, pooled sample and occurrence range methods are given in Figure 4.3. The environmental quality standards (EQS), i.e., the pH levels corresponding with a 5% reduction in species richness in case of acidification, were most stringent for the occurrence range method, followed by the pooled samples and quantile regression methods (Figure 4.4). However, the difference in EQS between the three methods was 0.3 pH unit at maximum.

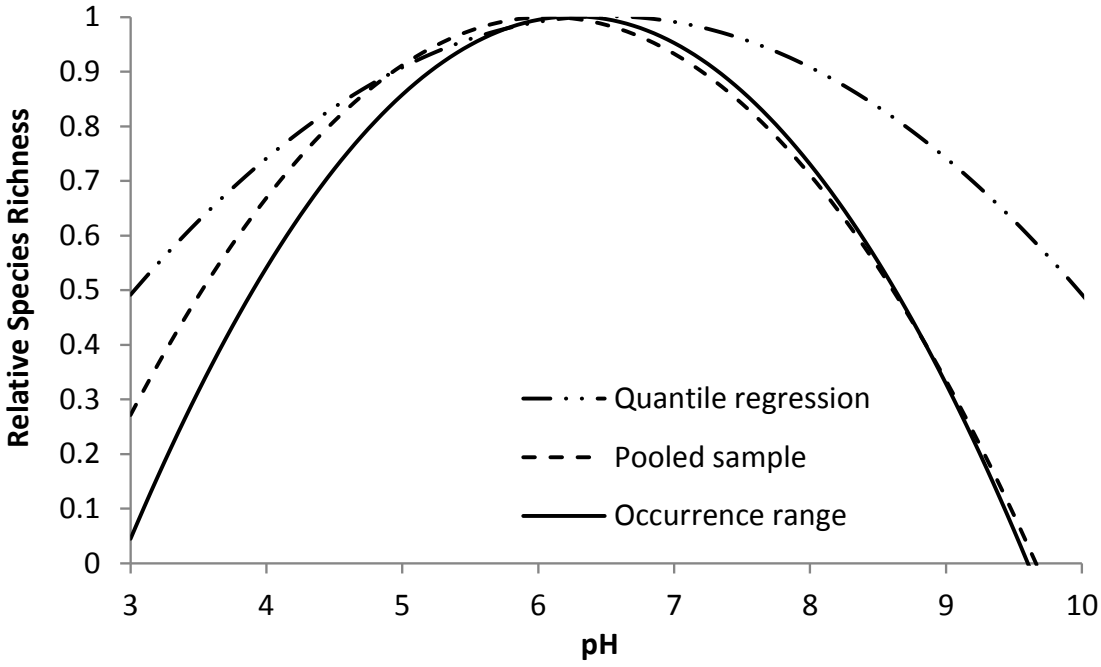

Figure 4.3. f-SSDs for the relative species richness (r-SR) along the pH gradient for the quantile regression (y = -0.75(-1.13- -0.31)+0.54(0.32-0.74)x-0.04(-0.06- -0.03)x^2), pooled sample (y = -1.94(-2.32- -1.22)+0.94(0.65-1.12)x-0.08(-0.10- -0.05)x^2), and occurrence range methods (y = -2.55(-3.16- -1.89)+1.12(0.94-1.32)x-0.03(-0.10- -0.01)x^2). The 95th percentile confidence intervals of the regression coefficients are given between brackets.

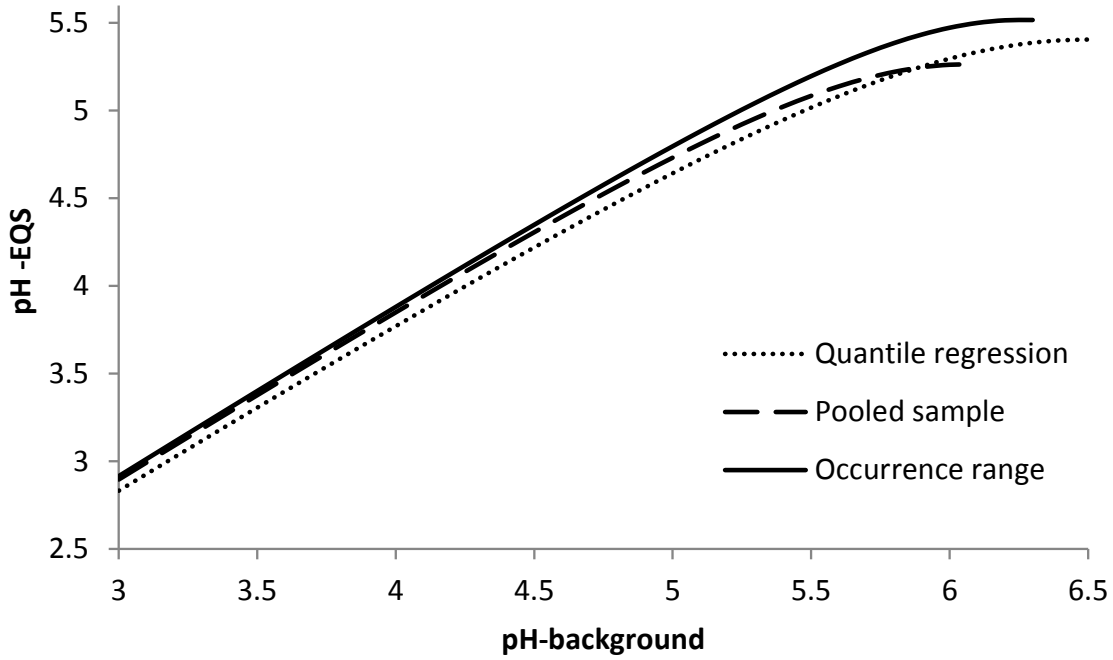

Figure 4.4. Environmental quality standards for pH (pH-EQS) corresponding to the respective background levels (pH-natural background) for each method.

Sensitivity analysis

Sensitivity of the quantile regression method to changes of the chosen quantile was tested by comparing response curves based on the 95th, 97.5th and 99th quantiles (Figure S4.1; Table S4.1). The response curves gave similar results, with a maximum pH of 6.5 irrespective of the quantile. The estimated SR in the pooled sample method depends on the number of samples used to derive the SACs and ranged from a maximum SR of 32 for one sample to a maximum SR of 601 for an infinite number of samples (Figure S4.2). At the extremes of the pH range, however, the differences in estimated SR are smaller, ranging from 13 to 122 at pH 3.5 and from 12 to 123 at pH 9. In the occurrence range method the width and maximum estimated SR is determined by the percentile that is used to derive the species occurrence ranges (Figure S4.3). The width ranged from 4.6 for the 100th to 3.6 for the 90th percentile. The maximum estimated SR ranged from 633 for the 90th to 721 for the 100th percentile.

4.4 Discussion

Interpretation

Each of our response curves suggests a unimodal relationship between the species richness of grassland vegetation and pH (Figure 4.1). The unimodal response is in line with the results of other studies, with comparable optimum pH and shape of the curve (Azevedo et al., 2013; Chytrý et al., 2010; Olsson et al., 2009; Wamelink et al., 2005). This suggests that the relationship between species richness and pH was successfully extracted from the field data. We derived the response curves specifically for grassland vegetation, as different vegetation types respond differently to changes in pH (Wamelink et al., 2005). Response curves with multiple optima may result from a dataset including multiple vegetation types (Figure S4.4), suggesting that response curves are preferably derived per vegetation type. However, response curves based on species richness do not account for changes in species composition that may occur within a vegetation type, because the number of species may remain the same along a particular environmental gradient, whereas species composition may change due to species replacements. Such species replacements may explain why the pooled samples method does not reveal major changes in species richness at intermediate pH levels (Figure 4.1). Species replacements along the pH gradient may also explain why the maximum number of species as obtained with the occurrence range method (702) is smaller than the total number of species in the dataset (1321). Apparently, there are no

pH values that are within the occurrence range of all the species, and the gradient in pH values is large enough to encompass multiple non-overlapping tolerance ranges of individual species.

As expected, the quantile regression method estimated species richness close to the maximum species richness observed at the sampling sites, i.e. the maximum realized species richness (Figure 4.1; Table 4.2). The maximum realized species richness was around 50 at intermediate pH values. Because the quantile regression curve is directly derived from the species richness observed in the field sites, this method in particular may be sensitive to the size of the relevés, as bigger plot sizes may lead to higher species richness. Furthermore, the quantile regression method may be particularly sensitive to underestimated species richness due to false absence records, for example because some plant species may not have germinated yet at the moment the relevé was recorded. However, the surface area of the relevés is chosen to obtain a representative picture of the species composition and richness of the respective vegetation type, and sites are generally visited during the growing season, when most species are present and visible (Schaminee et al., 1995).

At intermediate pH values, the potential species richness as derived with the pooled samples and occurrence range methods was about 10 to 14 times larger than the maximum realized species richness (Figure 4.1). Potential species richness estimates were higher for the occurrence range method than for the pooled samples method. This difference is found because in the occurrence range method a species is assumed to occur anywhere between its minimum and maximum pH value, irrespective whether it was actually observed at the pH values in between, whereas in the pooled samples method a species needs to be actually observed in a particular pH interval in order to contribute to the potential species richness.

The shapes of our response curves suggest that acidification would result in greater reductions in potential than in maximum realized plant species richness (Figure 4.1). This result is in line with two recent studies that concluded that large-scale declines of species richness are not necessarily accompanied by biodiversity loss at local scales (Dornelas et al., 2014; Vellend et al. 2013).

Management implications

For each response curve method we derived a field-based Species Sensitivity Distribution (f-SSD) (Figure 4.3). SSDs are typically derived for toxicants, generally based on a limited number of species tested in laboratory exposure experiments (Van Straalen & Denneman, 1989). To conduct laboratory experiment for all possible combinations of stressors and species, however, is almost impossible simply because there are so many (Azevedo et al., 2013; Cormier & Suter II, 2013a; Kefford et al., 2012). SSDs based on field observation have therefore been proposed instead, as these include the actual species pool and relevant environmental stressors of a particular area (Leung et al., 2005). The resulting f-SSDs can be used (1) to derive environmental quality standards for a particular environmental factor, and (2) to estimate relative changes in species richness along a specific environmental gradient (Posthuma et al., 2002). However, in contrast to anthropogenic toxicants, pH is a natural environmental factor, with varying natural background levels and ecological communities adapted to these levels (Wamelink et al., 2005). In order to account for this natural variation, we derived EQS based on a 5% reduction of the species richness corresponding with a given natural background pH (Figure 4.4), rather than a 5% reduction of the overall maximum species richness, as is common practice in EQS setting. EQS were slightly more stringent for potential than for maximum realized species richness, thus reflecting that acidification would result in larger declines of the former. However, differences in EQS between the three methods were only small and EQS varied mainly in relation to the natural background pH. Hence, in the derivation of EQS for pH it is much more important to consider intrinsic spatial differences in soil pH than methodological differences between f-SSD approaches.

Chapter 5

Context-dependent environmental quality standards of soil nitrate for terrestrial plant communities

Thomas M.W. J. van Goethem

Aafke M. Schipper

Wieger G.W. Wamelink

Mark A.J. Huijbregts

Submitted

Abstract

Environmental quality standards (EQS) are typically derived based on laboratory data. Here, a procedure is proposed to derive field-based EQS that are conditional on confounding environmental factors. To illustrate the procedure, a dataset was used with species richness observations of grasslands and forests and accompanying soil nitrate and pH measurements collected from 981 sampling sites in the Netherlands. Species richness was related to soil nitrate (NO_3) and acidity (pH) with quantile regression allowing for interaction effects. The resulting regression models were used to derive EQS for nitrate, quantified as the concentrations corresponding with a species richness equal to 95% of the species pool, for both grasslands and forest and for different pH levels. The EQS for NO_3 varied between 1.8 and 64.6 mg/kg with different pH levels (between pH of 4-9). The results further showed that the EQS based on red list species richness were a factor of 2 lower than those based on overall species richness. The results indicate that both natural background pH conditions and red list species are important factors to consider in the derivation of EQS for NO_3 for terrestrial ecosystems.

Key words: Context dependency; Grasslands; Forests; Interaction effects; Nitrate; pH; Quantile regression; Red list species; Species richness

5.1 Introduction

Environmental quality standards (EQS) specify the maximum permissible concentration or level of a specific environmental stressor (Van Straalen & Denneman, 1989; Van Goethem et al., 2015). The permissible level is generally set to be protective for 95% of the species pool (Kefford et al., 2011; Cormier & Suter II, 2013). EQS are used to evaluate at which places unacceptable ecological risks may occur due to the stressor of concern. EQS have mainly been established for chemical substances in the aquatic ecosystems and are typically derived on the basis of lab toxicity tests (Posthuma et al., 2002). There are, however, also possibilities to derive EQS on the basis of field data (Kefford et al., 2011; Schipper et al., 2014). The use of field data to derive EQS has the potential to significantly increase the ecological validity of EQS compared to the use of lab data. Yet, the field-based approach to derive EQS for a particular stressor requires that confounding effects of other stressors are adequately accounted for. As shown by Van Goethem et al. (2015), quantile regression can be used for this purpose. Quantile regression based on one of the upper boundaries of the response variable distribution (e.g. the 0.90 or 0.95 quantile) is expected to show the constraints imposed by the explanatory environmental variable of concern (Cade & Noon, 2003, Iwasaki & Ormerod, 2012).

The ecological relevance of field-based EQS may be further increased by accounting for possible interactions between the stressor of concern and other environmental factors. Soil nitrate and pH are considered to be important environmental factors determining plant species composition and richness (Pausas & Austin, 2001; Bobbink et al., 2010; Azevedo et al., 2013; Mueller et al., 2013). Several studies identified the influence of acidification and base cation depletion on plant responses to nitrogen deposition and suggest that sensitivity to nitrogen addition is co-determined by soil pH (Clark et al, 2007; Horswill et al., 2008). Hence, the response of species richness to nitrate is likely influenced by potential interaction effects with pH, implying that the EQS for NO_3 may at least partly depend on background pH conditions (Bobbink et al., 1998; Bobbink et al., 2010). This type of interaction has, however, not been included in the derivation of EQS up to now.

The aim of the present study was to derive field-based EQS for soil nitrate, accounting for pH as potentially influencing factor. EQS for soil nitrate were derived for grasslands as well as forests, based on either the overall plant species pool or the red list species

only. The dataset used in this analysis comprised presence-absence observations of plant species along gradients of soil pH (3-10) and NO_3 (0.01-210 mg/kg), collected from 981 sampling sites of grassland and forest vegetation across the Netherlands (Wamelink et al., 2012). To derive the EQS, quantile regression models were established relating species richness to soil pH and NO_3, thereby accounting for interaction effects between the factors. The resulting regression models were subsequently used to derive EQS for soil NO_3 conditional on soil pH, with the EQS quantified as the NO_3 concentration corresponding with 95% of the maximum species richness found along the gradient.

5.2 Methods

Data set

Monitoring data for grasslands and forests were selected from the ecological conditions (EC) database compiled by Wamelink et al. (2012). The dataset comprises 505 grassland and 592 forest vegetation relevés from the Netherlands, each accompanied by a soil NO_3 and pH measurement. The vegetation relevés were made according to the Braun-Blanquet method and were classified based on the vegetation classification of Schaminée et al. (1995) using the software tool Associa (Van Tongeren et al., 2008). A description of the selected grassland vegetation types is given in the supporting information (Table S5.1). Several relevés were repeat visits. To reduce potential confounding effects of temporal autocorrelation, only the most recent relevé per site was included in the dataset, leading to a decrease in the number of grassland relevés from 505 to 469 and forest relevés from 592 to 512. Per relevé, both the overall species richness and the red list species richness were determined. Red list species were identified using the red list species database from FLORON (2012) (Table S5.2). The characteristics of the dataset used are given in Table 5.1.

Table 5.1. Characteristics of the grassland and forest datasets, describing both overall and red list species richness (SR) of the relevés and the measured soil pH and NO_3 concentrations.

	Grassland			**Forest**		
	SR	*pH*	*NO_3 (mg/kg)*	*SR*	*pH*	*NO_3 (mg/kg)*
Min	4.0	3.9	0.1	1	3.4	0.1
0.05	9.0	4.3	0.2	7	3.8	0.7
0.5	23	5.6	4.2	24	5.0	11.9
0.95	47	8.3	64	46	8.3	101
Max	61	9.0	366	65	9.0	805

Species richness response models

Before model construction, the NO_3 measurements were 10 log transformed and the pH and NO_3 measurements were standardized to zero mean and unit variance. Quantile regression was used to relate the 95^{th} percentile of the species richness observations for grasslands and forest, and their respective red list species, to the soil NO_3 and pH measurements. Three models were constructed: a linear model, a Gaussian model and a baseline model where species richness is estimated by a constant (i.e., an intercept-only model). Because unimodal responses of grassland species richness have been observed for NO_3 (Bobbink et al., 2010), quadratic terms were added as potential predictors in the regression modelling. All possible combinations of predictors were fitted with interaction terms, whereby the quadratic predictor terms were only allowed if the linear term was included as well, in order to obtain models insensitive to linear transformations of the predictors (Nelder, 2000). The most parsimonious model was selected based on the Bayesian Information Criterion (BIC) (Lee et al., 2013). The same procedure was also done for NO_3 only as a reference model. The 95% confidence intervals of the regression lines were calculated based on the covariance matrix of the regression parameter estimates (Koenker, 1994). Model building and averaging was performed with the quantreg and MuMIn packages in R (Koenkers et al., 2013).

Environmental quality standards

EQS for NO_3 conditional upon soil pH natural background levels were derived using the quantile regression models based on NO_3 and pH for both overall and red list plant species richness in respectively grasslands and forests. These context-dependent EQS were derived for pH levels ranging from 4 to 9 in steps of 1 pH unit, thus covering the range of pH values in the dataset (Table 5.1). The pH-dependent EQS for NO_3 was defined as the NO_3 concentration corresponding with a 5% reduction of the maximum species richness at a given pH background level. The 5% reduction is in line with the way EQS are derived in the field of chemical risk assessment (see Posthuma et al., 2002).

In addition, as a reference EQS for soil NO_3 only were derived using the NO_3 regression models. The EQS was defined as the NO_3 concentration corresponding with a 5% reduction of the maximum species richness. These EQS do not account for the possible interaction with pH.

5.3 Results

Species richness response models

Species richness (SR) was estimated in relation to NO_3 only (Figure 5.1) and pH and NO_3 combined (Figure 5.2) for grasslands, forest and their respective red list species. Full model descriptions, including coefficients and 95% confidence intervals, are given in the SI (Table S5.3).

The regression models for NO_3 only showed positive unimodal responses along the nitrate gradient (Figure 5.1). Optimum NO_3 values were found in the range of 4.8 to 7.1 mg/kg, depending on the vegetation type (forest vs. grassland) and species pool (all species vs. red list species only) (Table 5.2). The response curves differed in their width and amplitude, where width was defined as the NO_3 range at 75% of the maximum species richness (SRmax) and amplitude as the relative difference between the maximum and minimum species richness estimated along the NO_3 gradient (Table 5.2). The widths ranged from 32 mg/kg for the grasslands red list species to 251 mg/kg for forests. The amplitude ranged from 0.27 for forests to 1.0 for both grassland and forest red list species.

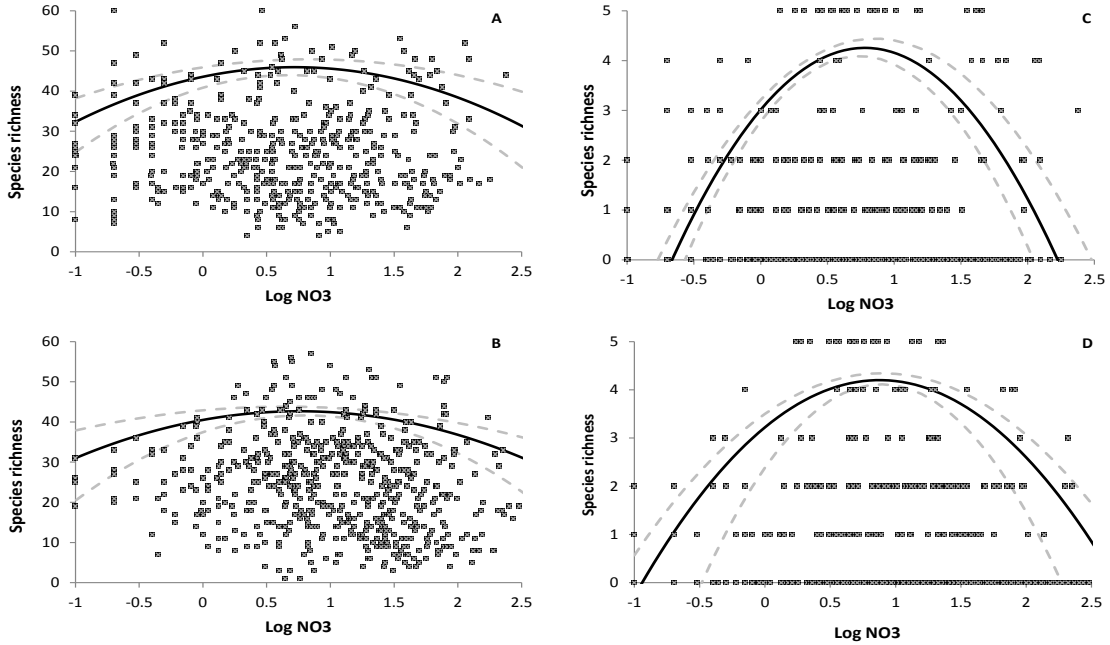

Figure 5.1. NO_3 species richness response curves for grasslands (A), forests (B), grassland red list species (C) and forest red list species (D). The response curves were derived with quantile regression based on the 95th quantile. Observed SR is plotted in gray, dotted lines indicate the 95% confidence intervals.

The regression models for NO_3 with pH interaction included also showed positive unimodal responses (Figure 5.2). Interaction between nitrate and pH was indicated by the regression coefficient for the interaction term (Table S5.3). Irrespective of vegetation type and species pool, the NO_3 concentration corresponding with the highest species richness (NO_3-optimum) decreased with increasing pH, up to a factor of 42 along the pH range (Table 5.2). The same was observed for the widths of the response curves, which decreased up to a factor of 17 from low to high pH, i.e. the slope of the response curve was steepest at highest pH levels (Figure 5.2, Table 5.2).

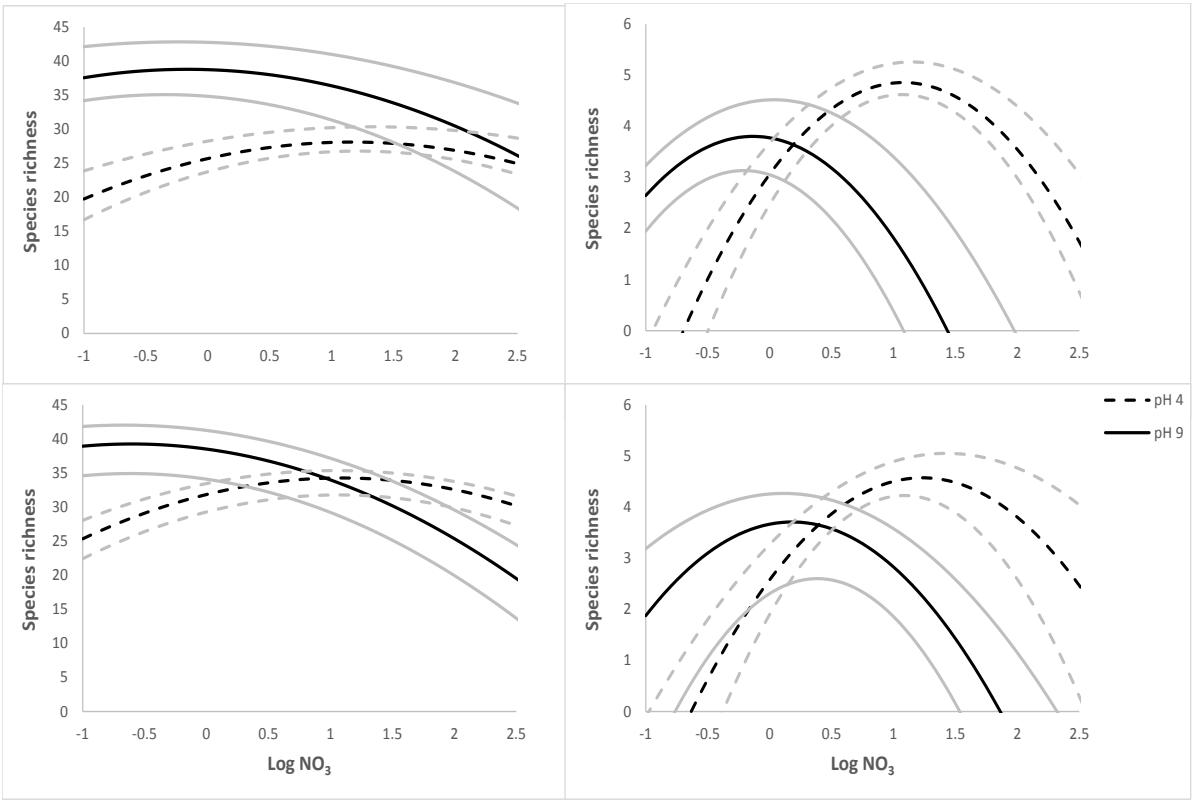

Figure 5.2. NO_3 species richness response curve conditional on background pH conditions (pH 4 and 9) for grasslands (A), forests (B), grassland red list species (C) and forest red list species (D). The response curves were derived with quantile regression based on the 95th quantile, grey lines indicate the 95% confidence intervals.

Table 5.2. Characteristics of the grassland and forest response curves for both all species and red list species. NO_3 at optimum SR ($NO3_{opt}$ in mg/kg), the NO_3 range at 75% of SR (width; in mg/kg) and amplitude (RSR) of the species richness response curves. Reference is based on the regression for NO_3 only, the pH curves based regression model for NO_3 and pH combined

	Grassland						Forest					
	All species			Red list species			All species			Red list species		
	$NO3_{max}$	Width	Amplitude	$NO3_{opt}$	Width $RSR_{0.75}$	Rel amp	$NO3_{opt}$	Width $RSR_{0.75}$	Rel amp	$NO3_{opt}$	Width $RSR_{0.75}$	Rel amp
Reference	4.8	190	0.29	6.3	32	1.0	5.6	251	0.27	7.1	60	1.0
pH 4	16	316	0.30	12.6	88	1.0	12.7	316	0.26	17.0	139	1.0
pH 5	7.9	316	0.18	6.3	51	1.0	5.0	316	0.16	10.2	84	1.0
pH 6	4.0	316	0.15	4.0	29	1.0	2.5	316	0.22	6.0	55	1.0
pH 7	2.5	316	0.19	2.5	16	1.0	1.3	218	0.28	3.8	32	1.0
pH 8	1.3	316	0.24	1.3	9	1.0	0.5	104	0.37	2.0	17	1.0
pH 9	0.8	141	0.33	0.8	5	1.0	0.3	35	0.51	1.4	10	1.0

Environmental quality standards

Environmental quality standards for nitrate (EQS-NO_3) are given in table 5.3. EQS based on the regression models for NO_3 only ranged from 12.6 for red list grassland to 32.9 mg/kg for all forests. Given the 95% confidence intervals, the EQS-NO_3 of grassland and forest species were not different from each other, while the EQS-NO_3 for red list species richness was found to be more stringent compared to the EQS based on overall species richness.

Accounting for the interaction between NO_3 and pH, the EQS-NO_3 become pH-dependent. The EQS-NO_3 was a factor of 1.4-2.5 higher at pH 4 compared to the pH-independent EQS-NO_3. In contrast, for pH 9 the EQS-NO_3 was a factor of 1.6-7 lower (more stringent) compared to the pH-independent EQS-NO_3 (Table 5.3).

Table 5.3. NO_3 environmental quality standards (in mg/kg) conditional on background pH conditions for grasslands, forest and their respective red list species. EQS reference refers to the standard

EQS-NO_3 (mg/kg)	**Grasslands**		**Forests**	
	All species	Red list	All species	Red list
Reference	26.3 (17.8-49.5)	12.6 (8.3-16.4)	32.9 (23.4-42.7)	17.2 (11.4-24.1)
pH 4	64.6 (42.6-84.0)	29.5 (19.5-38.4)	44.7 (31.3-62.6)	42.2 (27.9-59.1)
pH 5	56.2 (38.2-67.4)	17.0 (11.6-23.8)	39.8 (27.9-59.7)	26.3 (16.8-34.2)
pH 6	46.8 (28.1-65.5)	12.3 (8.6-17.2)	33.1 (21.2-53.0)	17.0 (10.5-27.2)
pH 7	30.2 (18.7-45.3)	5.2 (3.1-6.8)	25.7 (17.0-43.7)	9.5 (6.3-11.4)
pH 8	24.0 (14.4-38.4)	3.2 (2.2-5.1)	17.8 (12.1-24.9)	6.0 (4.1-7.8)
pH 9	16.6 (11.6-28.2)	1.8 (1.2-2.2)	13.2 (7.9-19.8)	3.5 (2.5-4.2)

derived based on the NO_3 only regression model. Between brackets the 95% confidence intervals.

5.4 Discussion

Species richness response curves

The response curves for NO_3 and pH combined and NO_3 only both suggest a unimodal relationship between plant species richness and nitrate (Figures 5.1 and 5.2). The unimodal response can be explained by the fact that oligotrophic conditions limit the occurrence of species due to nutrient limitation, while eutrophic conditions can lead to an increase in productivity and a subsequent decrease in species diversity due to competitive interactions (Goldberg et al., 1990; Bobbink et al., 2010, Ashmore et al., 2011). Furthermore, the response curve for NO_3 and pH combined suggest that in alkaline conditions the maximum species richness occurs at lower NO_3 levels and an increase in NO_3 beyond the optimum results in a stronger species richness decline. These patterns might be explained by the influence of pH on nitrogen cycles in soils (Bobbink et al., 2010; Bolan et al., 2003). Nitrification is reduced below pH 6 and is almost absent below pH 4.5, resulting in decreased NO_3 uptake and accumulation of NH_4 (Alexander, 1977; Bolan et al., 2003; Marschner, 1995; Bobbink et al., 2010). Some plant species may use NH_4 as a source of nitrogen, whereas for others the accumulated NH_4

may reach toxic concentrations (Alexander, 1977; Bolan et al., 2003). This may result in a shift in species composition towards species that primarily rely on ammonium as a nitrogen source (Bobbink et al., 2010; Bolan et al, 2003; Van den Berg et al., 2005). Species assemblages occurring at low pH conditions are therefore potentially less sensitive to changes in NO_3 concentration, resulting in a gentler slope of the response curve and a maximum species richness at higher NO_3 concentrations. In alkaline soils, on the other hand, NH_4 is nitrified more rapidly due to an increase in the activity of microorganisms involved in nitrification (Lyngstad, 1992; Puttanna et al., 1999). Alkaline conditions may therefore favor species that are specialized in the uptake of NO_3 as nitrogen source (Zvereva et al., 2008; Tinsley, 1973; Adams, 1986). These species assemblage are potentially more sensitive to changes in NO_3 as there is high resource competition between these efficient nitrate consumers, resulting in a steeper curve and maximum species richness occurring at lower NO3 concentrations (Fargione & Tillman, 2006).

Environmental quality standards

According to the EQS derived in our study, grasslands and forest are approximately equally sensitive to an increase in nitrate (Table 5.3). This is in line with other studies, as many semi-natural grassland and forest communities in the Netherlands, often dominated by species with low nutrient requirements, are sensitive to eutrophication (Dise et al., 2011; Gall et al., 2015). Red list species appeared to be more sensitive to eutrophication than plant species in general, according to the more stringent EQS. In general, red list species generally have narrower tolerance ranges compared to common species, especially for nutrients (Spitale, 2012; Wamelink et al., 2014). An increase in soil nitrate concentration is therefore more likely to lead to a decrease in red list species. By deriving environmental response curves and EQS for specific species groups, sensitive species groups can be identified and better protected (see also Azevedo et al., 2014; Del Signore et al., 2015).

For 15% of the grassland plots (26% for red list species) and 24% of the forest plots (42% for red list species) in our dataset, measured nitrate concentrations exceeded the respective EQS based on NO_3 only. However, the EQS for NO_3 conditional on pH suggest a more nuanced situation. For instance, based on a characteristic pH of 4.5 (Schaminée et al., 1995), fen meadows (Cirsio dissecti-Molinietum) have an EQS of 60.1 mg/kg NO_3, implying that measured NO_3 concentrations would exceed the EQS in only 3% of the fen

meadow plots. Similarly, hay meadows (Arrhenatheretum elatioris) have a characteristic pH of 7.5 (Schaminée et al., 1995) hence an EQS of 26.9 mg/kg NO_3, which corresponds to exceedance of the EQS in only 2% of the plots. Hence, although both vegetation types are considered grasslands, they have clearly different EQS for NO_3. This suggests that natural background pH conditions are an important factor to consider in the derivation of EQS for NO_3.

Chapter 6

How to unravel community assembly processes along environmental gradients? Comparing patterns of taxonomic and functional plant diversity along a pH gradient in grasslands

Thomas M.W.J. van Goethem

Mark A J. Huijbregts

Nils M. van Rooijen

G.W. Wieger Wamelink

Joop H.J. Schaminée

Aafke M. Schipper

Submitted

Abstract

Although there is compelling evidence that changes in soil acidity cause changes in plant community composition, the consequences of these changes for plant functional diversity have not yet been quantified. In this study taxonomic and functional diversity of plant communities in grasslands were quantified and compared in relation to soil pH. A dataset was used with plant species abundance and accompanying soil pH measurements collected from 1,087 sampling sites in the Netherlands. For each sampling site taxonomic and functional diversity were quantified based on three metrics each, reflecting richness and evenness. Functional diversity metrics were based on three plant traits representing the leading dimensions of ecological variation among plant species: specific leaf area, maximum plant height and average seed weight. Next, functional and taxonomic diversity metrics were related to pH with quantile regression and compared to null models representing random community assembly. Along the pH gradient, opposing patterns of taxonomic and functional diversity were found. Taxonomic diversity was highest at intermediate pH levels, whilst functional diversity increased towards both ends of the gradient, thereby differing from the null model expectations. The opposing patterns of taxonomic and functional diversity are not in line with environmental filtering and niche divergence hypotheses alone, suggesting a role for niche convergence and facilitation in the communities studied. Further, the results indicate that taxonomic and functional richness provide complementary information about community assembly and that a simultaneous analysis of both may help reveal the prevalence of different assembly processes.

Keywords: Community assembly, Diversity patterns, Environmental filtering, Facilitation, Niche convergence, Niche divergence, Plant traits, Quantile regression, Soil pH

6.1 Introduction

Understanding how community assembly processes influence diversity patterns across environmental gradients is central to the study of plant community ecology (Kraft et al. 2011; Spasojevic & Suding, 2012). Community assembly is assumed to result from abiotic environmental factors and biotic interactions that influence the composition and structure of communities at local scales (Chesson, 2000; Schwilk & Ackerly, 2006). A common hypothesis suggests that environmental filtering increases species similarity, because successful species are likely to share similar trait values, leading to functional convergence (Petchey et al. 2007). Competitive interactions between species, on the other hand, are expected to prevent coexisting species from being too similar through niche divergence (MacArthur & Levins 1967; Chesson, 2000). Based on these hypotheses, it is expected that functional diversity is relatively low in habitats with strong abiotic stress and relatively high in habitats with more benign environmental conditions, where competitive interactions between species are relatively strong (Weiher & Keddy 1995). Functional diversity is defined here as the value and range of species traits in niches space that influence their performance and thus ecosystem functioning (Diaz and Cabido, 2001).

Recent studies, however, have proposed additional community assembly processes, with different outcomes in terms of functional diversity (Chesson, 2000; HilleRisLambers et al., 2012). For instance, the coexistence hypothesis postulates that competitive interactions can lead to functional convergence instead of functional divergence (Chesson 2000; Grime 2006). Functional convergence can occur when a limited number of traits have a superior performance in given environmental conditions, leading to niche convergence (Chesson 2000; HilleRisLambers et al., 2012). Further, the facilitation hypothesis states that facilitative interactions between species in stressful environments may result in increased functional diversity instead of a decrease, as these interactions often involve functionally distinct species (Butterfield, 2009; Callaway et al., 2002; Callaway 2007).

There is compelling evidence that changes in soil acidity cause changes in plant community composition (Azevedo et al., 2013; Bellard et al., 2012; Pausas and Austin, 2001). Acidic soils (pH 3-6) are generally characterized by increased mobilization of metals and decreased nitrification and organic matter decomposition, while alkaline soils (pH 7.5-9) are characterized by decreased macro-nutrient solubility (Bobbink et al.,

2010). Both acidification and alkalinisation of soils may lead to species-specific reduced biomass and root growth and reduced germination (Zvereva et al., 2008). Understanding the effects of changes in soil pH on both taxonomic and functional diversity in plant communities, independently of other environmental drivers, can help to improve the understanding of community assembly processes (Lehmann et al., 2002). A number of studies have related functional diversity in plant communities to specific environmental gradients (Mason et al. 2012; Spasojevic et al., 2014; Walker and Chapin, 1986). For soil pH, however, responses of functional diversity have not yet been systematically quantified.

In this paper taxonomic and functional plant diversity in grasslands were quantified in relation to pH. Several studies have shown that species richness is highest at intermediate pH levels (Azevedo et al., 2013; Van Goethem et al., 2014). The aim of this study was to explore whether functional diversity follows the same response to pH as taxonomic diversity, which would be consistent with the classic hypotheses of environmental filtering and niche divergence assembly processes. The dataset used in the analysis comprises terrestrial plant species abundance and pH collected from 1,087 sampling sites across the Netherlands (Wamelink et al., 2012). For each sampling site, taxonomic diversity and functional diversity were quantified with three metrics each. The functional diversity metrics were calculated based on three plant traits representative of three leading dimensions of ecological variation among terrestrial vascular land plant species (Westoby et al., 2002; Wright et al. 2005), i.e., maximum plant height, specific leaf area (SLA) and average seed weight.

6.2 Methods

Monitoring data

The ecological conditions (EC) database, compiled by Wamelink et al. (2012), comprises vegetation relevés from the Netherlands (Schaminée et al. 2012), each accompanied by a soil pH value measured in H_2O. The database contains 5,243 grassland relevés, covering the period from 1936 to 2011. The vegetation relevés were made according to the Braun-Blanquet method, recording plant species and their abundance occurring in 1 to 2 m^2 of vegetation, and were classified based on the vegetation classification of Schaminee et al. (1995-1999) using the software tool Associa (Van Tongeren et al., 2008). Several relevés were repeat visits; the dataset included 109 sites with 2 to 10 relevés and 32 sites with more than 10 relevés. To reduce potential bias induced by

temporal autocorrelation, only the most recent relevé from each site was included in the dataset used for this study, leading to a decrease in the number of releves from 5,243 to 4,412. Furthermore, following the conclusions from Pakeman (2014), only those relevés were selected where at least 95% of the total abundance was represented by species with trait data available for all three traits (see below). This resulted in a decrease from 4,412 to 1,087 relevés, with a pH range between 3.3 and 8.9.

Trait data

Plant traits were selected to represent three leading dimensions of ecological variation among terrestrial vascular plant species: leaf, seed and height dimensions (Westoby et al., 2002; Wright et al. 2005). These dimensions are strongly linked to key aspects of both the established and regenerative phases of plants (Lavorel et al., 2012). Leaf, seed and height dimensions were represented by, respectively, specific leaf area (SLA in mm^2/mg), average seed weight (mg) and plant potential maximum height (m) (Westoby et al., 2012). Plant trait values were standardized to zero mean and unit variance, to ensure that each trait had the same weight in the diversity indicators (Casanoves et al., 2011). The species-specific trait data were collected from the LEDA Traitbase, which is a publicly available database with plant traits of the flora of Northwest Europe (Kleyer et al., 2008).

Biodiversity metrics

Three metrics of taxonomic diversity were calculated: species richness (SR), diversity (H) and evenness (H_E). Species richness was calculated as the total number of species. Species diversity was calculated with the Shannon-Wiener index, which is a composite metric of richness and evenness (Magurran, 1988; Rosenzweig, 1995). Species evenness was calculated with the Shannon equitability metric (H_E), which normalizes the Shannon-Wiener index for the number of species, thus quantifying evenness independent of the number of species (Purvis & Hector 2000; Tuomisto, 2010).

The framework proposed by Villeger et al. (2008) and Laliberte & Legendre (2010) was followed to quantify three functional diversity metrics: functional richness (FRich), functional evenness (FEve) and functional divergence (FDiv) (Mason et al., 2005; Villéger et al., 2008). Functional richness is the amount of niche space filled by species in the community, functional evenness quantifies the evenness of the abundance distribution in filled niche space and functional divergence describes the degree to which the

abundance distribution in niche space maximizes divergence in functional traits within the community (Petchey & Gaston, 2006).

Per relevé, the six metrics were calculated using only those species for which trait data was available. Functional diversity indicators were calculated with the FD package in R (Laliberté et al., 2014). Equations used to calculate the taxonomic and functional diversity metrics are given in the supplementary information (Text section S6.1).

Relating taxonomic and functional diversity to pH

The values of each biodiversity metric were standardized to zero mean and unit variance in order to facilitate comparison of the responses along the pH gradient. Then, quantile regression was used to relate the standardized biodiversity metrics to pH. Quantile regression has been proposed as an approach to quantify the relationship between a biotic response and a single environmental factor from field monitoring data, because quantile regression based on one of the upper boundaries of the response variable distribution (e.g. the 0.90 or 0.95 quantile) is expected to show the constraints imposed by the explanatory environmental variable of concern (Cade & Noon, 2003, Iwasaki & Ormerod, 2012; Lancaster and Belyea, 2006, Van Goethem et al., 2015). For each biodiversity metric, three models were constructed at the 90% quantile (Cade, 2003; Visser & Sasser, 2009): a baseline model where the response variable is estimated by a constant (i.e., an intercept-only model), a linear model ($y = \beta_0 + \beta_1 \cdot x$) and a Gaussian model ($y = \beta_0 + \beta_1 \cdot x + \beta_2 \cdot x^2$). The most parsimonious model was selected based on the Bayesian Information Criterion (Lee et al., 2013). Higher level models were not included as the literature available did not give indications of multimodal responses to pH. The 95% confidence intervals of the regression lines were calculated based on the covariance matrix of the regression parameter estimates (Koenker, 1994). The quantile regression was performed with the quantreg package in R (Koenkers et al., 2013).

Null models

The patterns in taxonomic diversity along the pH gradient were compared with those expected for randomly distributed taxonomic diversity along the pH gradient. A null model was calculated for each taxonomic diversity metric by randomizing the pH measurements among the relevés, thereby conserving the species composition within each relevé. The pH values were randomized 1,000 times and quantile regression was applied to relate each taxonomic diversity metric to pH for each of the 1,000 trials. Then,

the results from the 1000 trials were averaged to establish the null models for the taxonomic diversity metrics.

The patterns in functional diversity along the pH gradient were compared with null models that assume random trait composition (Weiher & Keddy, 1995; Spasojevic et al., 2012). For a given functional diversity metric, the null model represents the level of functional diversity that can be expected by chance. A null model for each functional diversity metric was calculated by conserving the number and abundances of the species and the pH within the relevés and randomizing the identity of the species, selecting from the entire species pool, so that the trait composition within the relevés was assembled by random chance. The identity of the species in the relevés was randomized 1,000 times and for each of the 1,000 randomizations, a quantile regression model was established to relate each functional diversity metric to pH. Again, the results were averaged over the 1000 trials to establish the null models.

6.3 Results

Response curves for taxonomic diversity

The pH response curves for taxonomic diversity all had positive unimodal shapes (Figure 6.1, Table S6.1). The response curves differed with respect to the optimum pH, which ranged from 5.3 for H_e to 6.3 for SR. The amplitude, defined as the relative difference between maximum and minimum modelled value of the respective response variable within the observed pH-range, was smallest for H_e and largest for SR. The 95% confidence intervals confirmed the postive unimodal shapes of the response curves, except for H_e, where a linear response to pH cannot be excluded. The same response shapes were found with quantile regression models based on the 75^{th} or 95^{th} quantile instead of the 90^{th} quantile, suggesting that the unimodal patterns are robust with respect to the chosen quantile (Figure S6.1). Taxonomic richness and diversity were lower than the null model at the extremes of the pH gradient, and equal to or slightly higher than the null model at intermediate pH levels.

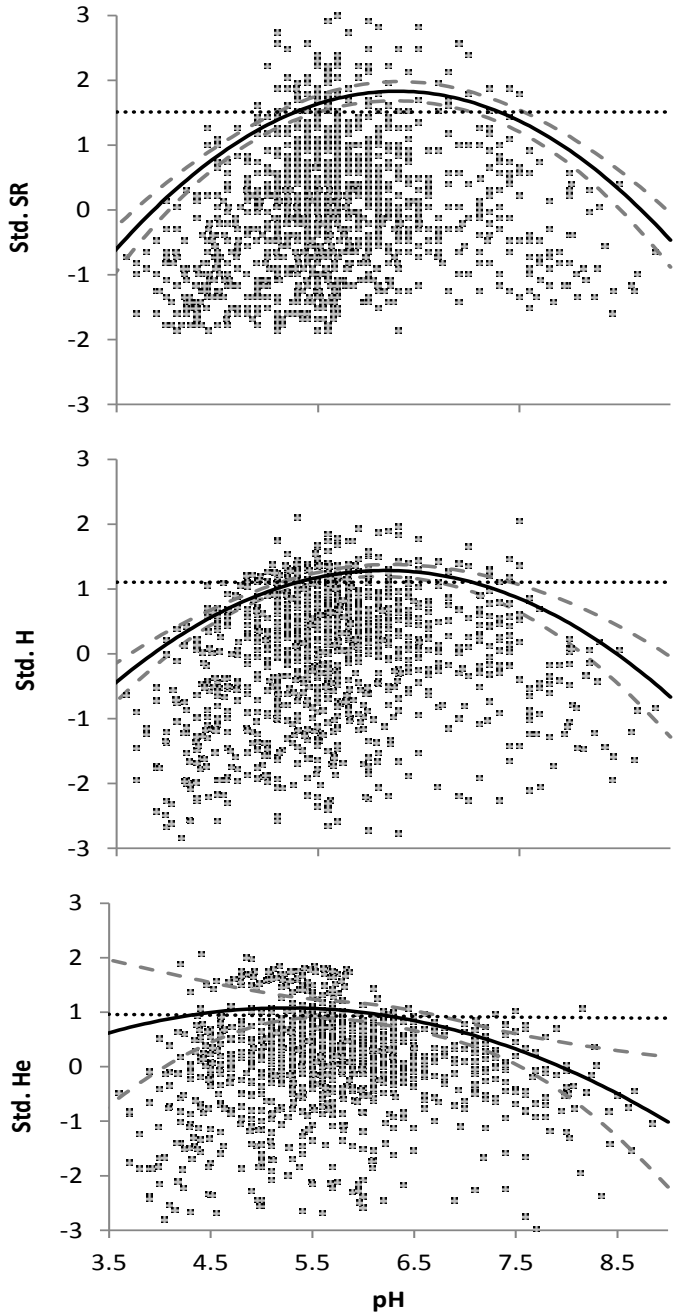

Figure 6.1. pH response curves for standardized species richness (upper), species diversity (middle) and species evenness (bottom). The 95% confidence interval is indicated with the dotted lines. The y-axis has steps of 1 standard deviation above or below the mean. The horizontal line represents the null model assuming random taxonomic composition.

Response curves for functional diversity

The response curves for functional richness, evenness and divergence all showed a negative unimodal response along the pH gradient (Figure 6.2, Table S6.1). The pH corresponding with the lowest functional diversity ranged from 5.8 for FEve to 6.4 for

FRic. The amplitude was smallest for FEve and largest for FRic. The 95% confidence intervals confirmed the negative unimodal shapes of the response curves. Response curves derived with different quantiles showed the same shapes (Figure S6.2). At the extremes of the pH gradient, functional diversity metrics exceeded the null model expectations, while at intermediate pH levels the functional diversity was similar to or slightly lower than predicted by the null model (Figure 6.2).

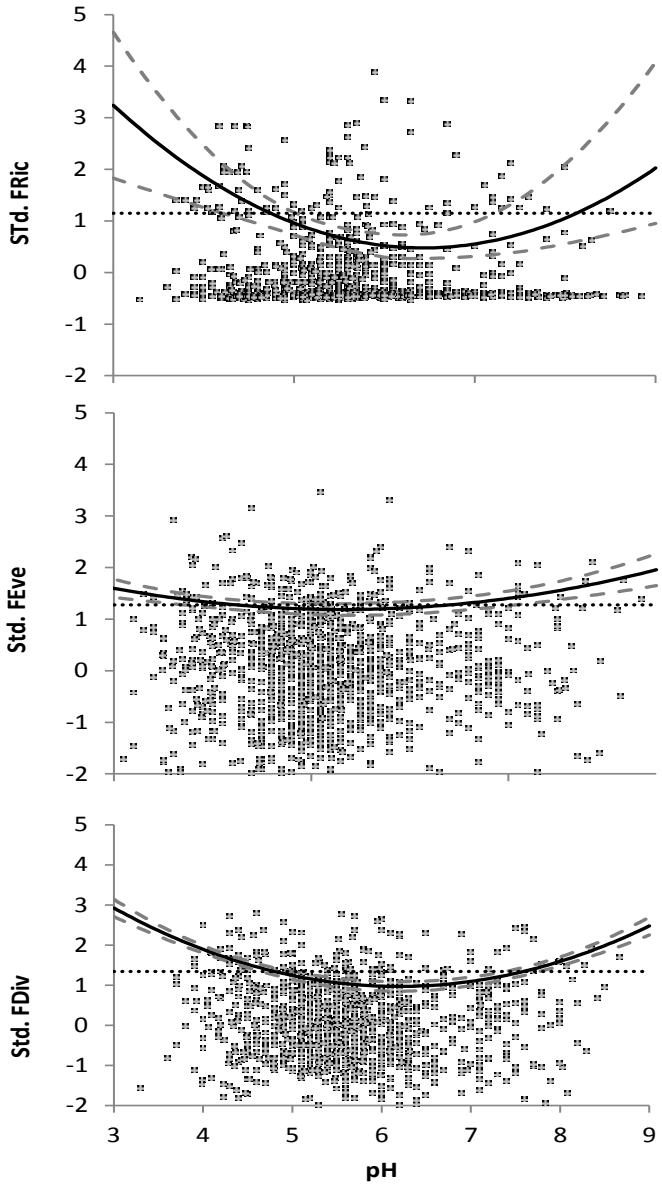

Figure 2. pH response curves for standardized functional richness (upper), evenness (middle) and divergence (bottom). The 95% confidence interval is indicated with the dotted lines. The y-axis has steps of 1 standard deviation above or below the mean. The horizontal line represents the null model assuming random trait composition.

6.4 Discussion

Patterns of taxonomic and functional diversity

Functional diversity was lower than expected based on the null models at intermediate pH, while taxonomic diversity was higher than the null model predictions. The opposite pattern was observed at the ends of the pH gradient, i.e. higher than expected functional diversity and lower than expected taxonomic diversity. The results for taxonomic diversity are in line with findings from other studies, and can be explained by the fact that fewer species are adapted to acidic or basic conditions (Azevedo et al., 2013; Van Goethem et al., 2014; Lee, 1999). The results for functional diversity can be explained by analyzing the changes in species composition along the pH gradient (Text section S6.2). At intermediate pH levels the plant assemblages are dominated by competitive grasses (Table S6.2, S6.3 and S6.4). These grasses form dense vegetation where it is difficult for other plant species, such as tree species, to germinate. As a result FD is low, because grasses are functionally similar. At both ends of the pH gradient, the assemblages tend to contain more tall-growing species compared to intermediate pH, resulting in higher FD. On a single trait level it is also observed that there is a shift to more tall-growing species, as the community weighted mean values of maximum plant height and seed weight were also highest at the ends of the pH gradient (Figure S6.2).

We found that the pattern of opposing responses along the pH gradient was particularly visible for taxonomic and functional richness. Taxonomic and functional evenness change much less along the pH gradient. This might be explained by the fact that these metrics are not based on the identity of the species/traits but on the relative distribution of their abundances. Note, however, that trait plasticity was not taken into account in the analysis, as no quantitative data on intraspecific trait variation in relation to pH was available for the set of species used in the analysis. Several studies have shown that seed weight and SLA may decrease when plants are exposed to environmental stress (Kichenin et al., 2013; Marshall et al., 1986; Matthies, 1990). This could lead to lower estimates of functional diversity at the ends of the pH gradient, where environmental stress is highest (Cordlandwehr et al., 2013; Mitchell & Bakker et al., 2014). Functional richness in particular may change because in this metric abundance is not taken into account (Jung et al., 2014). However, variation in trait values due to plasticity are expected to be small relative to the large intrinsic trait differences between plant species (Kichenin et al., 2013).

Prevalence of different community assembly processes

Patterns of functional diversity have been used as a proxies for identifying community assembly processes (Mason et al., 2011; Mouchet et al., 2010). It is, however, challenging to disentangle such processes by using patterns of functional diversity only, as trait distribution patterns may reflect a combination or cancelling out of different community assembly processes (Chesson, 2000; Mayfield et al. 2005). For instance, lower than random functional diversity might be caused by both environmental filtering and niche convergence, while higher than random functional diversity may reflect facilitation or niche divergence processes (Mayfield & Levine 2010; HilleRisLambers et al., 2012; Cavender-Bares et al., 2009). However, environmental filtering and facilitation are expected to occur in harsh environmental conditions, which allow relatively few species to exist (i.e., relatively low taxonomic diversity), while both niche convergence and divergence are expected to result from biotic interactions in more benign environmental conditions, where more species can occur (i.e., relatively high taxonomic diversity). This suggests that the prevalence of different community assembly processes might be assessed by contrasting taxonomic richness and functional richness patterns while comparing each to their respective null models. By simultaneously analyzing the complementary information of the SR and FRic patterns, four distinct combinations of lower/higher than random SR and FRic can be identified (Table 6.1).

Table 6.1. *Schematic overview of the prevalence of different community assembly processes.*

		Functional richness	
		Higher than random	**Lower than random**
Taxonomic richness	**Higher than random**	Niche divergence	Niche convergence
	Lower than random	Facilitation	Environmental filtering

The opposing patterns of species richness and functional richness as found in this study are in contrast to results expected based on classic environmental filtering and niche divergence hypotheses (Weiher & Keddy 1995; Cornwell et al., 2006; Mouillot et al., 2013). The lower than random functional richness and higher than random taxonomic

richness at intermediate pH values may be explained by niche convergence, i.e. all taxa that do not possess the near optimal trait are eliminated (Chesson, 2000; HilleRisLambers et al., 2012). If all species are similarly fit, it becomes harder for any one species to outcompete others (Chesson, 2000). Higher than random functional richness combined with lower than random taxonomic richness at the ends of the pH gradient can be caused by facilitation processes, which allow coexistence for a few species in stressful environments and generally involve functionally distinct species, hence leading to higher functional diversity (Callaway, 2007; Spasojevic & Suding, 2012).

Conclusions

Quantile regression was used to analyse functional diversity patterns in grassland vegetation along a pH gradient. The results showed opposing patterns of taxonomic and functional diversity of plant communities along the pH gradient. Taxonomic and functional diversity indices clearly provide complementary information about community assembly and a simultaneous analysis can help to reveal the prevalence of different assembly processes. Here, our results point in the direction that facilitation and niche convergence might be considered important assembly processes to shape grassland communities in relation to soil pH.

Chapter 7

Synthesis

7.1 Introduction

This thesis aimed to develop and apply species sensitivity distributions (SSDs) to quantify the effects of ozone formation, acidification and eutrophication on taxonomic and functional characteristics of terrestrial plant communities. To that end, the following specific goals were addressed:

(1) to cover a representative species pool,

(2) to include more environmental factors,

(3) to apply novel, assemblage-level endpoints.

In the preceding chapters, laboratory-based SSDs have been established and applied to set environmental quality standards (EQS) and to assess environmental risk for tropospheric ozone on terrestrial plant communities (chapter 2). These SSDs have also been applied in the context of life cycle impact assessment (chapter 3). Further, field-based SSDs (f-SSDs) have been established and used to set EQS for pH (chapter 4) and NO_3 (chapter 5) and to understand biodiversity patterns along a pH gradient by deriving functional endpoints (chapter 6). The present chapter first evaluates the extent to which f-SSDs have proven able to address the three goals of the thesis (section 7.2), using the differences between field- and laboratory-based SSDs as a starting point for the discussion (Table 7.1). Next, the applicability of lab- and field-based SSDs is evaluated and compared (section 7.3). The chapter concludes with implications and recommendations for the further development and application of SSDs (section 7.4).

Table 7.1. Comparison of different characteristics of field- and laboratory-based species sensitivity distributions.

	Laboratory	**Field**
Stressors	Mainly toxicants	Mainly non-toxic environmental factors
Input data	Mainly species-specific responses of survival, reproduction or growth	Species presence/absence, occurrence or abundance
Species	Mostly easily cultured test species	Species pool of a specific area
Method(s) to fit the curve	Logistic or log-normal cumulative distribution functions	Quantile regression, species accumulation curves, stacked species distributions
Ecological context	No	Yes

7.2 Potential benefits of field-based SSDs

Representative species pool

SSDs based on laboratory data are typically derived based on a few easily cultured species, while the f-SSD approach includes information on the actual species pool (Leung et al., 2005; Cormier & Suter II, 2013). Both lab- and field-based SSDs can be derived for specific species groups or vegetation types (see chapter 2, 5; De Hoop et al., 2011; Verbrugge et al. 2012). It may be relevant to derive SSDs for specific species groups, as they can differ strongly in their sensitivity to environmental stressors (Leuven et al., 2011; Azevedo et al., 2013; Verbrugge et al. 2012). This variability is more easily captured with f-SSDs, as field surveys generally cover multiple geographic regions and large environmental gradients, while providing species observations on different organizational levels, i.e. species, community and ecosystem level (Ozinga, 2008). In chapter 5 differences in EQS between red list species and non-red list species were found, while in chapters 2 and 5, no significant differences in EQS were found between grasslands and forests f-SSDs for tropospheric ozone and nitrate (NO_3), respectively. This suggests that differences are found in some cases, but not in all (De Hoop et al., 2011; Verbrugge et al., 2012).

Lab-based SSDs typically reflect the cumulative distribution of species-specific responses on growth, reproduction and/or survival, while f-SSDs often use (relative) species richness as an indicator of species community response, either by aggregating the species present along the environmental gradient or directly relating species richness to the environmental factor of concern (Azevedo et al., 2013; Leung et al., 2005; Schipper et al., 2014). The different methods to derive f-SSDs (see chapter 4) can be used to assess the effects of environmental factors on biodiversity for different species pools, i.e. corresponding to either potential or realized species richness. Realized and potential species pools can be used as proxies for alpha diversity, measuring species diversity at a site, or gamma diversity, measuring species diversity regionally, respectively (Semeniuk & Cresswell, 2013). f-SSDs may therefore provide a tool to assess the extent to which biodiversity change in local assemblages contributes to global biodiversity loss, which is currently poorly understood (Dornelas et al., 2014; Fukami et al., 2013; Vellend et al., 2013).

Relevant (non-toxic) environmental factors

SSDs based on laboratory experiments are often limited by the (non-toxic) environmental factors for which data is available. Laboratory data for non-toxic stressors are especially scarce, as these types of environmental factors have not been considered in 'traditional' risk assessments (Posthuma et al., 2002) or because of practical and cost-efficiency reasons (Hayes et al, 2007; Ashmore, 2005). Field-based SSDs have been proposed not only because their assumed greater ecological relevance by covering the actual species pool, but also because the greater availability of field data for (non-toxic) environmental factors (Schipper et al., 2014; Wamelink et al., 2012). Field data, however, is often characterized by considerable scatter among the species observations, as a result of confounding environmental factors (Cade & Noon, 2003; Van den Brink et al., 2002). There are two options to deal with the confounders when deriving f-SSDs: either excluding confounding influences (chapter 4) or quantifying them (chapter 5). The influence of confounders can be quantified when field data includes measurements for multiple environmental factors (Schaminée et al., 1995; Wamelink et al., 2012; Schipper et al., 2014 Theoretically, lab-based SSDs can also be used to quantify confounding effects, by varying the levels of a confounding factor of interest in factorial exposure experiments. However, for data availability reasons, f-SSDs have more potential for quantifying confounding influences.

Novel assemblage-level endpoints

When species abundance is reported, field data can be used to derive both functional and taxonomic endpoints for SSDs (see chapter 6). Functional diversity (FD) might be considered a relevant endpoint for SSDs as it is recognized as an important indicator of community performance (Diaz and Cabido, 2001; Villéger et al., 2008; Mori et al., 2013). Further, SSDs based on functional diversity endpoints can be used to analyse and understand fundamental ecological processes, such as community assembly (chapter 6). However, uncertainty due to trait plasticity in relation to specific environmental factors needs to be addressed. Until now, however, almost no field data is available that includes site-specific trait values (Ozinga, 2008; Cordlandwehr et al, 2013). It may also be difficult to determine which relevant causal mechanism exist between traits and environmental factors (Cordlandwehr et al., 2013). These issues need to be addressed to successfully interpret and apply SSDs based on FD, to ensure that the predicted relationship between FD and the environmental factor of interest is valid in actual field conditions. Furthermore, the applicability of f-SSDs with FD endpoints is currently limited to analysing ecological processes along environmental gradients. For application in environmental risk assessment or to derive EQS, meaningful policy targets for FD need to be determined first.

7.3 Applicability of field- and laboratory-based SSDs

SSDs can be applied for different purposes (see chapter 1): deriving EQS, environmental risk assessment, LCIA and biogeographical research (Sijm et al., 2002; Posthuma et al., 2008). Lab-based SSDs may best be used to set an EQS for new, toxic substances, as the methodology to derive generic and uniform EQS for substance testing is widely accepted and well documented (Van Straalen & Denneman, 1989; Raimondo et al., 2007; Posthuma & De Zwart, 2012). Additionally, field data for new, toxic substances is generally not available. For all other purposes, however, f-SSDs may best be used. First, f-SSDs can more easily account for both background conditions and other confounding environmental factors, which can have considerable influence on EQS. For instance, in chapter 4, natural background pH values appeared highly influential on the EQS for acidification. In chapter 5, it was shown that background pH also influenced the EQS for eutrophication. Further, f-SSDs can more easily assess the sensitivity of specific species groups (see chapter 2, 5). Hence, the f-SSD approach enables to assess environmental risk for specific sets of conditions and species. Finally, f-SSDs are more appropriate to

quantify taxonomic and functional characteristics of species communities in relation to specific environmental factors across biogeographical regions.

Several conditions should be met, however, when deriving and applying f-SSDs. First, the dataset used to derive an f-SSD should include representative references conditions, i.e. the environmental gradient should include non-disturbed conditions. This is important because when a data set only includes observations from an already degraded system, the baseline may have potentially shifted. For example, an EQS based on monitoring data of an already polluted area may prove to be very lenient, as added stress does not result in added damage (Huijbregts et al., 2011). Determining the appropriate reference level for (non-toxic) environmental factors is therefore important when assessing environmental risk. Second, the methods for deriving f-SSDs require sufficient observations of both species or species assemblages and co-occurring environmental conditions (Iwasaki & Ormerod, 2012; Kefford et al., 2012). Unfortunately, there are no standardized protocols for measuring environmental characteristics and reporting in databases (Wamelink et al., 2012). For instance, in chapter 5, the number of data points for NO_3 was limited because the soil concentrations were measured with different methods. Also, it is not always clear whether the environmental factor was measured at the same site or time as the species observations. This is important to consider, as spatial and temporal variation in soil conditions can be large, even when sites are in short (time) distance of each other (Cain et al., 1999; Wamelink et al., 2012). f-SSDs based on mismatched data could result in spurious relationships between endpoint indicators and the environmental factor of concern.

Theoretically, with optimal data availability, f-SSDs for a particular environmental factor can be derived for each specific species group and set of site conditions. Although the results of this thesis suggest that it is important to consider background conditions, confounding factors and specific species groups, it remains to be further investigated what level of detail is actually required in deriving and applying f-SSDs.

7.4 Implications and future research recommendations

The research in this thesis aimed to develop and apply SSDs for terrestrial plant communities. Based on the results obtained, several conclusions as well as recommendations for future research have been identified, which are provided below.

7.4.1 Implications for developing and applying species sensitivity distributions

(i) Field surveys including species observations and measurements of co-occurring environmental factors are a valuable source of data for deriving SSDs (chapter 4-6).

(ii) SSDs for specific species groups might provide an important tool for protecting endemic, keystone or commercially valued species (chapter 2, 5).

(iii) Natural background conditions are important to consider in risk assessment (chapter 4, 5).

(iv) SSDs based on field data can be used for better understanding and quantifying biogeographical patterns along environmental gradients, for instance, by contrasting taxonomic and functional diversity patterns (chapter 6).

(v) Lab-based SSDs might primarily be used for deriving environmental quality standards for new, toxic substances, whilst f-SSDs can principally be used in environmental risk assessment, LCIA and biogeographical research.

7.4.2 Recommendations for future research

(vi) It is recommended to do a comparative study of field- and lab-based SSDs for the same environmental factors and species, to provide further insight in the benefits and limitations of the different approaches.

(vii) More research is needed into factors (e.g. traits) that could explain differences in sensitivity to environmental factors between species groups.

(viii) The selection of relevant traits for functional diversity endpoints is important. It should be further investigated which relevant causal mechanism exist between traits and environmental factors.

(ix) The influence of trait plasticity on SSDs for functional endpoints should be assessed by comparing trait data obtained from the lab with direct field measurements.

(x) It is recommended to derive policy-relevant reference conditions for functional diversity for the application in environmental risk assessment and to derive EQS.

Appendices

Table S2.1. List of perennial grassland species with their exposure-response functions (endpoint:biomass) based on OTC experiments. With y = ax + b, x: AOT40 in ppm h and n are the number of datapoints used in the regression. Species which exhibited no biomass decrease are bold.

	Species	Family	a	b	EC_{10} (ppm.h)	n	R^2
1	*Rumex acetosa*	Polygonaceae	-0.048	1.02	2.14	9	0.22
2	*Malva sylvestris*	Malvaceae	-0.047	1.06	2.28	-	0.63
3	*Vaccinium vitis-idaea*	Ericaceae	0.042	1.03	2.46	4	0.98
4	*Phleum alpinum*	Poaceae	-0.04	1.14	2.84	18	0.8
5	*Leontodon hispidus*	Asteraceae	-0.031	0.97	3.18	5	0.49
6	*Cirsium arvense*	Asteraceae	-0.03	0.99	3.24	5	0.04
7	*Phleum pratense*	Poaceae	-0.027	1.09	3.96	55	0.46
8	*Dianthus deltoides*	Caryophyllaceae	-0.024	0.97	3.98	3	1
9	*Trifolium subterraneum*	Fabaceae	-0.026	1.08	4.14	3	0.46
10	*Campanula rotundifolia*	Campanulaceae	-0.021	1.02	4.98	4	0.07
11	*Valeriana officinalis*	Valerianaceae	-0.016	1	6.46	4	0.67
12	*Nardus stricta*	Poaceae	-0.012	0.99	8.3	4	0.73
13	*Trifolium repens*	Fabaceae	-0.011	0.94	8.76	6	0.89
14	*Hieracium pilosella*	Asteraceae	-0.011	0.97	8.78	5	0.06
15	*Silene acaulis*	Caryophyllaceae	-0.009	0.94	10.2	3	0.52
16	*Chrysanthemum leucanthemum*	Asteraceae	-0.009	1.01	11.26	6	0
17	*Lychinis flos-cuculi*	Caryophyllaceae	-0.013	1.5	11.38	10	0.52
18	*Holcus lanatus*	Poaceae	-0.009	1	11.52	8	0.6
19	*Festuca rubra*	Poaceae	-0.008	1	12.5	8	0.22
20	*Tragopogon orientalis*	Asteraceae	-0.007	1.01	14.26	4	0.85
21	*Cirsium dissectum*	Asteraceae	-0.007	0.96	14.5	4	0.09
22	*Hypochaeris radicata*	Asteraceae	-0.006	0.95	15.02	5	0.54
23	*Centaurea jacea*	Asteraceae	-0.006	1.01	15.76	6	0.74
24	*Trifolium pratense*	Fabaceae	-0.007	1.09	15.82	10	0.8
25	*Taraxacum officinale*	Asteraceae	-0.006	1.08	17.96	4	0.37
26	*Poa pratensis*	Poaceae	-0.005	0.96	18.48	9	0.06
27	*Dactylis maritima*	Poaceae	-0.005	0.91	18.92	9	0.27
28	*Eupatorium cannabinum*	Asteraceae	-0.005	1.07	19.78	4	0.72
29	*Briza media*	Poaceae	-0.005	0.99	21.5	5	0.75
30	*Anthoxanthum odoratum*	Poaceae	-0.004	0.94	21.9	7	0.38
31	*Saxifraga cernua*	Saxifragaceae	-0.004	1.04	24.26	6	0.17
32	*Polygonum viviparum*	Polygonaceae	-0.003	1	29.32	3	0.99
33	*Achillea millefolium*	Asteraceae	-0.003	1.04	31.4	4	0.99
34	*Achillea ptarmica*	Asteraceae	-0.003	1.02	35.08	4	0.53
35	*Lotus corniculatus*	Fabaceae	-0.003	0.96	36.82	8	0.04
36	*Knautia arvensis*	Dipsacaceae	-0.003	1.02	40.88	4	0.58
37	*Deschampsia flexuosa*	Poaceae	-0.002	1.08	59.96	6	0.64
38	*Salvia pratensis*	Lamiaceae	-0.001	1.08	83.38	4	0.75
39	*Dactylis glomerata*	Poaceae	-0.001	0.9	150.42	3	0.34
40	*Plantago lanceolata*	Plantaginaceae	-0.001	0.96	192.58	17	0.96
41	***Phalaris arundinacea***	**Poaceae**	**0.027**	**0.89**	**-**	**3**	**0.76**
42	***Festuca pratensis***	**Poaceae**	**0.018**	**0.92**	**-**	**3**	**0.13**

43	***Anthyllis vulneraria***	**Fabaceae**	**0.017**	**0.98**	**-**	**5**	**0.72**
44	***Silene dioica***	**Caryophyllaceae**	**0.015**	**0.91**	**-**	**4**	**0.92**
45	***Silene vulgaris***	**Caryophyllaceae**	**0.014**	**0.97**	**-**	**3**	**0.83**
46	***Galium saxatile***	**Rubiaceae**	**0.011**	**0.91**	**-**	**4**	**0.05**
47	***Molinia caerulea***	**Poaceae**	**0.01**	**0.88**	**-**	**8**	**0.39**
48	***Salix herbacea***	**Salicaceae**	**0.008**	**1.07**	**-**	**4**	**0.98**
49	***Deschampsia caespitosa***	**Poaceae**	**0.008**	**1.01**	**-**	**6**	**0.83**
50	***Carex bigelowii***	**Cyperaceae**	**0.007**	**1**	**-**	**4**	**0.1**
51	***Saxifraga cespitosa***	**Saxifragaceae**	**0.006**	**0.92**	**-**	**6**	**0.15**
52	***Calluna vulgaris***	**Ericaceae**	**0.006**	**0.92**	**-**	**16**	**0.04**
53	***Alchemilla alpina***	**Rosaceae**	**0.005**	**0.96**	**-**	**3**	**0.25**
54	***Festuca ovina***	**Poaceae**	**0.005**	**1.04**	**-**	**7**	**0.03**
55	***Lolium perenne***	**Poaceae**	**0.003**	**0.98**	**-**	**6**	**0.1**
56	***Agrostis capillaris***	**Poaceae**	**0.003**	**1**	**-**	**4**	**0.84**
57	***Carum carvi***	**Apiaceae**	**0.002**	**0.96**	**-**	**4**	**0.03**
58	***Onobrychis viciifolia***	**Fabaceae**	**0.002**	**1.1**	**-**	**4**	**0.43**
59	***Arrhenatherum elatius***	**Poaceae**	**0.001**	**0.91**	**-**	**6**	**0.51**
60	***Trisetum flavescens***	**Poaceae**	**0.001**	**1.1**	**-**	**4**	**0.61**
61	***Bromus erectus***	**Poaceae**	**0.001**	**1.05**	**-**	**4**	**0.99**
62	***Alopecurus pratensis***	**Poaceae**	**0.001**	**0.96**	**-**	**5**	**0.24**

Table S2.2. List of annual grassland species with their exposure-response functions (endpoint:biomass) based on OTC experiments. With y = ax + b, x: AOT40 in ppm h and n are the number of datapoints used in the regression. Species which exhibited no biomass decrease are bold.

	Species	Family	a	b	EC_{10} (ppm.h)	n	R^2
1	*Trifolium striatum*	Fabaceae	-0.046	0.9	1.94	3	0.95
2	*Medicago minima*	Fabaceae	-0.049	0.97	1.98	3	0.98
3	*Trifolium angustifolium*	Fabaceae	-0.046	0.96	2.06	3	0.85
4	*Matricaria chamomilla*	Asteraceae	-0.051	1.06	2.08	-	0.16
5	*Papaver dubium*	Papaveraceae	-0.041	0.98	2.4	-	0.93
6	*Biserrula pelecinus*	Fabaceae	-0.031	0.88	2.84	3	0.21
7	*Trifolium cheleri*	Fabaceae	-0.023	0.81	3.48	3	0.77
8	*Lolium rigidum*	Poaceae	-0.021	0.89	4.26	3	0.5
9	*Trifolium glomeratum*	Fabaceae	-0.019	0.95	4.92	3	0.66
10	*Matricaria matricarioides*	Asteraceae	-0.021	1.1	5.3	-	0.25
11	*Avena sterilis*	Poaceae	-0.019	1.01	5.4	3	0.09
12	*Senecio vulgaris*	Asteraceae	-0.017	1.14	6.72	6	0.39
13	*Aegilops geniculata*	Poaceae	-0.013	0.96	7.6	3	0.51
14	*Bromus sterilis*	Poaceae	-0.009	0.98	10.92	3	0.19
15	*Chrysanthemum segetum*	Asteraceae	-0.008	0.96	11.96	3	0
16	*Chenopodium album*	Amaranthaceae	-0.007	0.94	13.28	6	0
17	*Briza maxima*	Poaceae	-0.006	0.87	13.82	3	0.08
18	*Bromus arvensis*	Poaceae	-0.006	1.03	16.3	3	0.01
19	*Poa annua*	Poaceae	-0.005	0.98	18.14	3	0.88
20	*Papaver rhoeas*	Papaveraceae	-0.005	0.91	19.06	3	0.78
21	*Bromus hordeaceus*	Poaceae	-0.004	0.98	23.34	3	0.7

22	*Crepis biennis*	Asteraceae	-0.002	1.08	67.36	4	0.04
23	***Agrostemma githago***	**Caryophyllaceae**	**0.006**	**1.05**	**-**	**3**	**0.83**
24	***Centaurea cyanus***	**Asteraceae**	**0.002**	**1.03**	**-**	**3**	**0.02**
25	***Aegilops triuncialis***	**Poaceae**	**0.002**	**1.04**	**-**	**3**	**0.81**

Table S2.3. List of tree species with their dose-response functions (endpoint:biomass) based on OTC experiments. With y = ax + b, x: AOT40 in ppm h.

	Species	Family	a	b	EC_{10} (ppm.h)	R^2
1	*Fraxinus excelsior*	Oleaceae	-0.0255	-	3.9	0.73
2	*Fagus sylvatica*	Fagaceae	-0.0174	-	5.74	-
3	*Betula pendula*	Betulaceae	-0.0099	-	10.06	-
4	*Pinus sylvestris*	Pinaceae	-0.0073	-	13.66	-
5	*Quercus faginea*	Fagaceae	-0.0073	-	13.66	0.57
6	*Quercus pyrenaica*	Fagaceae	-0.0061	-	16.38	0.57
7	*Quercus robur*	Fagaceae	-0.0056	-	17.74	-
8	*Quercus petraea*	Fagaceae	-0.0054	-	18.58	-
9	*Picea abies*	Pinaceae	-0.0028	-	35.72	0.65

Table S2.4. Probability on Equality of Means and Variances between the species groups (p-value).

	μ	σ
Annual-Perennial	**0.01**	0.92
Annual-Trees	0.1	**0.045**
Perennial-Trees	0.82	0.16

Table S2.5. Means (μ), standards deviations (σ) and HC_5 values in ppm.h (90% confidence interval) for annual grassland species and perennial grassland species at R2 cutoff values of 0, 0.5 and 0.75.

	Perennial species			Annual species		
R2 cutoff	**0**	**0.5**	**0.75**	**0**	**0.5**	**0.75**
n species	39	21	11	25	11	7
μ	1.14	1.19	1.20	0.84	0.72	0.62
σ	0.47	0.50	0.59	0.42	0.42	0.45
HC_5	2.33 (1.59-3.19)	2.22 (0.96-4.61)	1.58 (0.34-3.86)	1.37 (0.75-2.09)	1.03 (0.35-1.70)	0.70 (0.12-1.62)

[1]calculated for current critical levels of ozone

Table S3.1. Grouping of the land cover classes in forest, grassland species or not considered categories. Land cover types based on the Global Land Cover 2000 database (Bartholomé and Belward, 2005).

	Land cover classes	Grassland	Forest	Not considered
1	Tree Cover, broadleaved, evergreen		1	
2	Tree Cover, broadleaved, deciduous, closed		1	
3	Tree Cover, broadleaved, deciduous, open		1	
4	Tree Cover, needle-leaved, evergreen		1	
5	Tree Cover, needle-leaved, deciduous		1	
6	Tree Cover, mixed leaf type		1	
7	Tree Cover, regularly flooded, fresh water (& brackish)		1	
8	Tree Cover, regularly flooded, saline water,		1	
9	Mosaic: Tree cover / Other natural vegetation		1	
10	Tree Cover, burnt		1	
11	Shrub Cover, closed-open, evergreen	1		
12	Shrub Cover, closed-open, deciduous	1		
13	Herbaceous Cover, closed-open	1		
14	Sparse Herbaceous or sparse Shrub Cover	1		
15	Regularly flooded Shrub and/or Herbaceous Cover			1
16	Cultivated and managed area			1
17	Mosaic: Cropland / Tree Cover / Other natural vegetation			1
18	Mosaic: Cropland / Shrub or Grass Cover			1
19	Bare Areas			1
20	Water Bodies (natural & artificial)			1
21	Snow and Ice (natural & artificial)			1
22	Artificial surfaces and associated areas			1
23	No Data			1

Table S3.2. Emissions of NMVOC and NOx for each European region in 2010 (in metric ton) (Verstreng et al., 2012).

Region	NMVOC	NOx
Albania	35,848	27,941
Austria	151,740	159,875
Remaining N.E. Atlantic	31,004	862,809
Bosnia and Hercegovina	46,218	53,978
Baltic Sea	13,422	360,598
Belgium	150,390	232,194
Bulgaria	113,509	146,722
Black Sea	3,632	94,410
Belarus	262,338	270,864
Switzerland	98,595	71,249
Serbia and Montenegro	154,284	167,845
Cyprus	6,365	21,058
Czech Republic	156,896	187,082
Germany 1	222,389	258,295
Germany 2	182,534	203,296
Germany 3	205,488	213,637
Germany 4	446,200	506,921
Denmark	73,072	146,707
Estonia	30,139	27,553
Spain 1	285,200	351,936
Spain 2	99,711	96,627
Spain 3	90,171	106,764
Spain 4	210,432	317,019
Spain 5	104,831	97,968
Finland 1	99,287	116,357
Finland 2	25,107	34,915

France 1	155,202	159,371
France 3	115,018	127,154
France 4	139,813	145,824
France 5	260,909	300,723
France 6	136,175	131,449
France 7	204,960	224,898
United Kingdom 1	285,221	407,638
United Kingdom 2	271,345	165,432
United Kingdom 3	378,775	512,248
Greece	167,805	266,381
Croatia	104,939	94,298
Hungary	94,988	135,272
Ireland	54,521	99,000
Italy 1	534,336	541,743
Italy 2	460,874	464,316
Lithuania	48,660	41,165
Luxembourg	8,983	28,107
Latvia	24,394	28,919
Republic of Moldova	43,433	64,394
Mediterranean Sea	69,103	1,914,070
The FYR of Macedonia	32,118	40,576
Malta	2,209	5,877
Netherlands	213,227	315,350
Norway 1	30,083	64,844
Norway 2	92,215	128,113
North Sea	29,243	777,251
Poland1	142,307	188,553
Poland 2	146,004	221,905
Poland 3	129,721	205,084

Portugal	176,888	213,619
Romania	347,848	282,694
Russian Federation	2,564,563	2,515,522
Sweden 1	57,332	55,235
Sweden 2	162,697	145,240
Slovenia	29,695	39,372
Slovakia	66,696	72,108
Turkey	656,169	851,800
Ukraine 1	417,195	777,018
Ukraine 2	321,171	407,133

Table S3.3. Characterization factors for European regions in 2010. CFs for natural vegetation, for NMVOC and NOx, and for lognormal, average and linear. CFs in PAF.m^2.yr/kg.

	NMVOC			**NOx**		
Region	**Lognormal**	**Average**	**Linear**	**Lognormal**	**Average**	**Linear**
Europe*	**1.64**	**0.20**	**4.81**	**5.60**	**0.66**	**16.39**
Albania	1.35	0.17	2.75	11.91	1.35	23.25
Austria	2.50	0.25	5.98	12.20	1.06	29.13
Remaining N.E. Atlantic	0.76	0.10	2.45	2.04	0.28	7.32
Bosnia and Hercegovina	1.53	0.18	3.38	13.51	1.28	32.09
Baltic Sea	1.34	0.20	6.53	0.76	0.12	5.72
Belgium	2.92	0.37	9.19	2.30	0.18	6.73
Bulgaria	0.94	0.13	2.29	8.98	1.14	25.15
Black Sea	1.00	0.16	2.67	3.97	0.65	13.52
Belarus	0.78	0.13	4.01	2.28	0.44	20.55
Switzerland	3.06	0.29	6.53	16.42	1.45	31.57
Serbia and Montenegro	1.57	0.19	3.79	10.64	1.12	28.16
Cyprus	0.51	0.12	1.17	6.60	1.53	15.25
Czech Republic	2.22	0.25	6.63	5.79	0.60	18.25

Germany 1	1.88	0.25	7.11	3.24	0.44	13.93
Germany 2	2.00	0.29	8.19	1.49	0.22	6.53
Germany 3	2.98	0.35	9.61	1.45	0.18	4.14
Germany 4	3.39	0.34	8.78	7.89	0.74	19.28
Denmark	1.63	0.24	7.33	0.96	0.16	5.97
Estonia	0.40	0.06	2.23	1.13	0.20	13.72
Spain 1	2.51	0.26	5.35	8.16	0.92	18.30
Spain 2	1.39	0.18	3.89	6.16	0.80	18.35
Spain 3	1.30	0.18	3.07	9.20	1.20	21.58
Spain 4	1.24	0.15	4.11	4.18	0.49	14.97
Spain 5	0.78	0.10	2.07	10.99	1.48	30.74
Finland 1	0.34	0.05	2.59	0.73	0.16	9.07
Finland 2	0.30	0.05	1.48	1.08	0.22	9.37
France 1	1.62	0.21	5.22	5.01	0.67	14.59
France 3	2.31	0.24	6.01	13.27	1.38	31.75
France 4	1.39	0.16	3.59	12.64	1.44	35.13
France 5	2.44	0.24	5.08	20.56	1.88	39.88
France 6	2.36	0.28	7.15	3.51	0.43	9.51
France 7	2.09	0.26	6.70	4.72	0.57	12.91
United Kingdom 1	2.34	0.34	8.23	0.45	0.06	1.23
United Kingdom 2	1.27	0.18	4.21	2.18	0.44	9.62
United Kingdom 3	2.00	0.32	7.02	0.50	0.04	1.98
Greece	1.21	0.17	2.24	9.14	1.18	17.25
Croatia	2.77	0.27	5.75	14.80	1.19	29.75
Hungary	1.59	0.19	4.38	8.60	0.84	26.45
Ireland	1.28	0.21	4.45	2.27	0.46	8.94
Italy 1	2.78	0.28	4.91	11.40	1.00	18.00
Italy 2	5.06	0.40	8.96	12.95	0.98	21.42
Lithuania	0.65	0.10	2.87	2.40	0.43	19.86

Luxembourg	3.63	0.41	10.15	3.80	0.46	10.47
Latvia	0.64	0.10	3.04	1.83	0.33	17.69
Republic of Moldova	0.97	0.14	3.16	3.84	0.55	15.18
Mediterranean Sea	2.11	0.26	3.82	3.17	0.39	5.70
The FYR of Macedonia	1.37	0.18	2.98	9.82	1.18	22.41
Malta	2.36	0.31	4.01	1.30	0.24	2.78
Netherlands	2.69	0.36	8.62	-0.34	-0.05	-1.15
Norway 1	0.62	0.09	2.21	2.05	0.35	13.55
Norway 2	0.86	0.13	3.65	1.99	0.37	13.55
North Sea	2.26	0.33	8.17	0.48	0.07	2.02
Poland1	1.05	0.16	4.46	3.20	0.53	18.15
Poland 2	1.11	0.17	4.68	2.93	0.47	15.28
Poland 3	1.72	0.22	6.33	3.01	0.39	13.02
Portugal	1.40	0.17	4.40	4.10	0.54	13.52
Romania	0.99	0.14	2.99	5.56	0.72	24.47
Russian Federation	0.41	0.08	3.09	1.33	0.36	11.83
Sweden 1	0.30	0.05	1.42	1.62	0.28	12.42
Sweden 2	0.65	0.11	3.94	1.33	0.25	14.74
Slovenia	3.19	0.30	6.68	15.71	1.19	31.62
Slovakia	1.72	0.22	5.08	7.43	0.79	27.55
Turkey	0.76	0.15	1.75	5.82	1.27	13.62
Ukraine 1	0.92	0.17	3.81	1.68	0.37	7.54
Ukraine 2	1.02	0.16	3.94	3.59	0.59	18.16

*Emission weighted CF for Europe

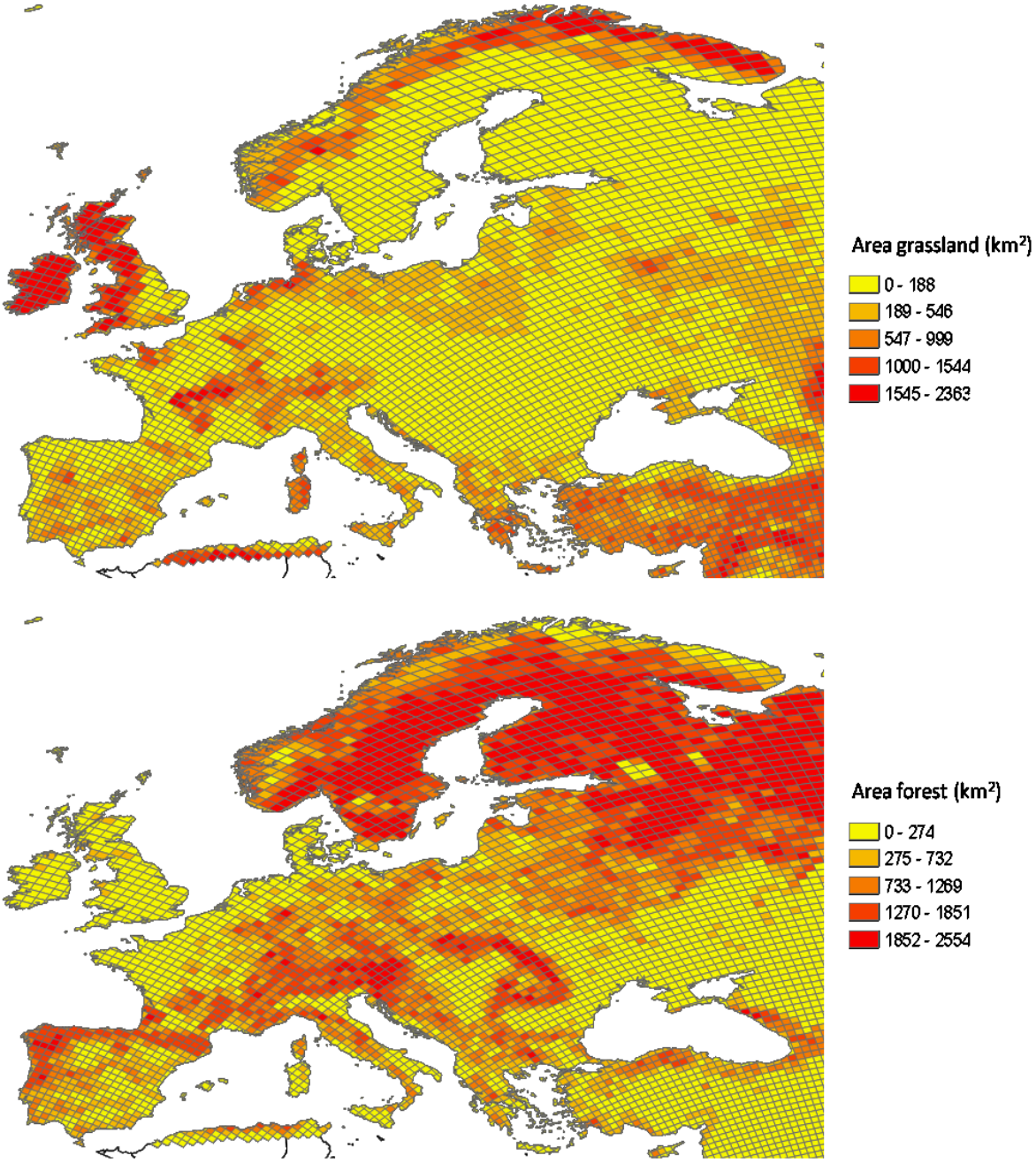

Figure S3.1. Area of grassland (a) and forest (b) in each grid (in km^2). Classification based on the GLC2000 database (Bartholomé, 2005).

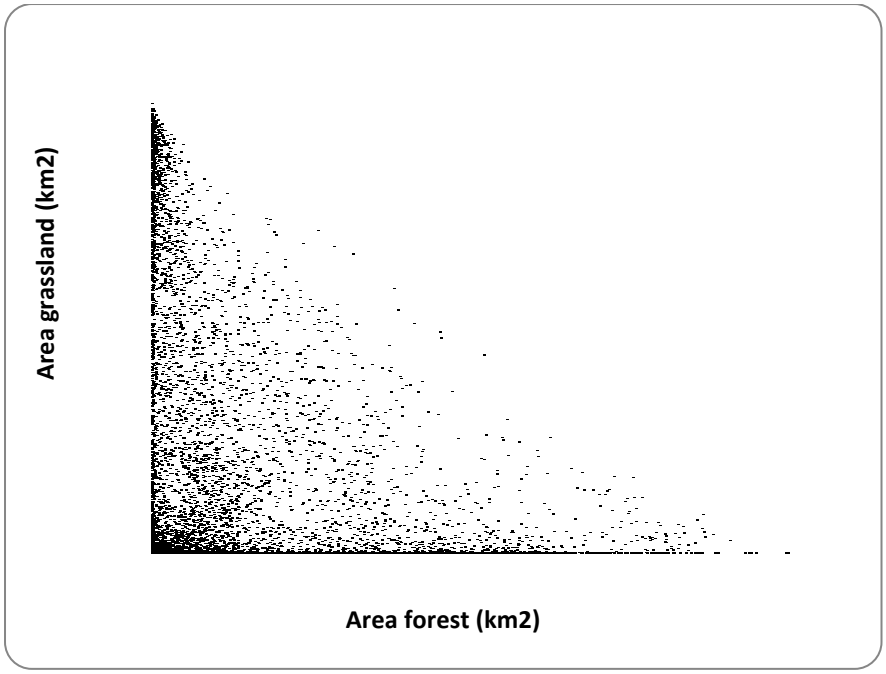

Figure S3.2. Comparison between the area covered by grassland and forest in each grid.

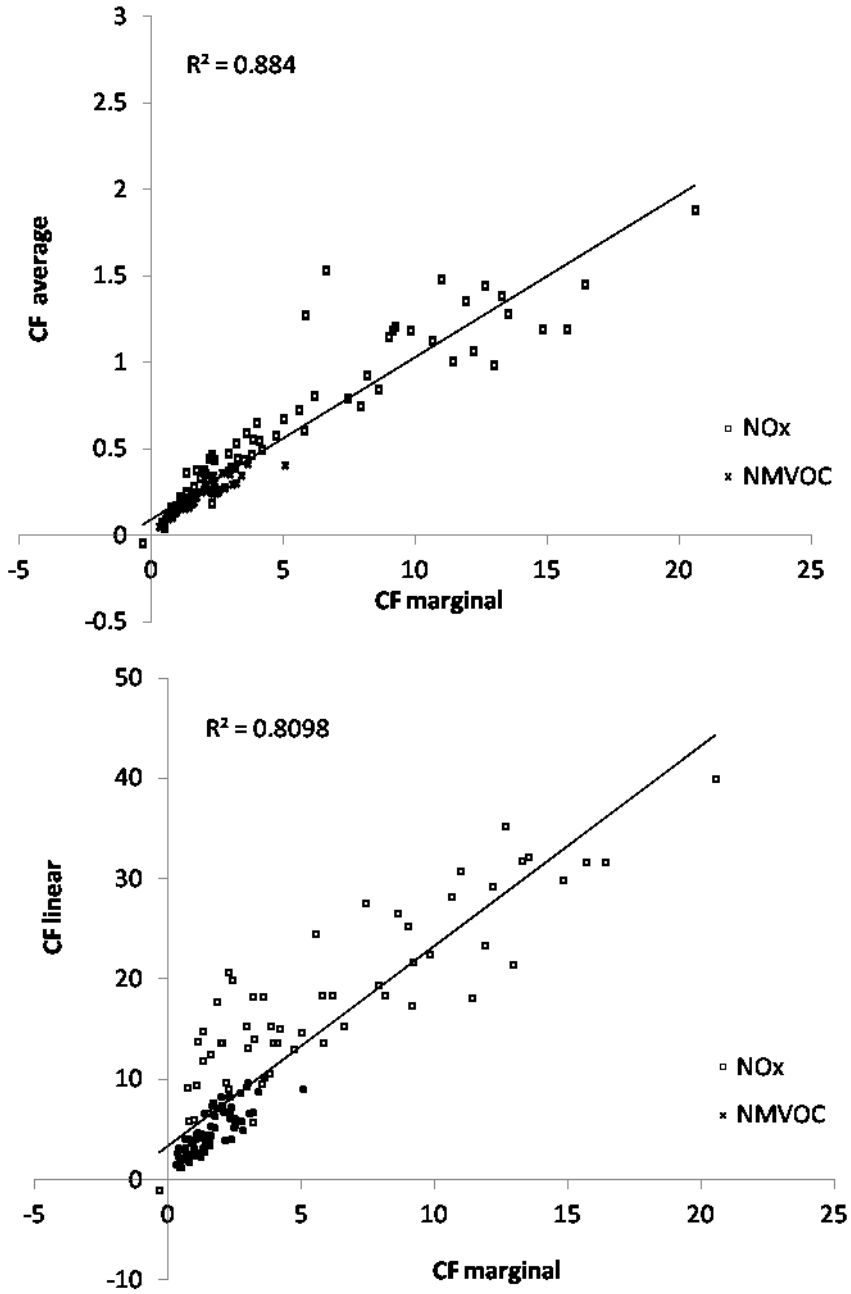

Figure S3.3. Correlation between the characterization factors based on the marginal and average effect factor (upper figure) and marginal and linear effect factor (lower figure). Both r-values were reported with a p-value <0.0001.

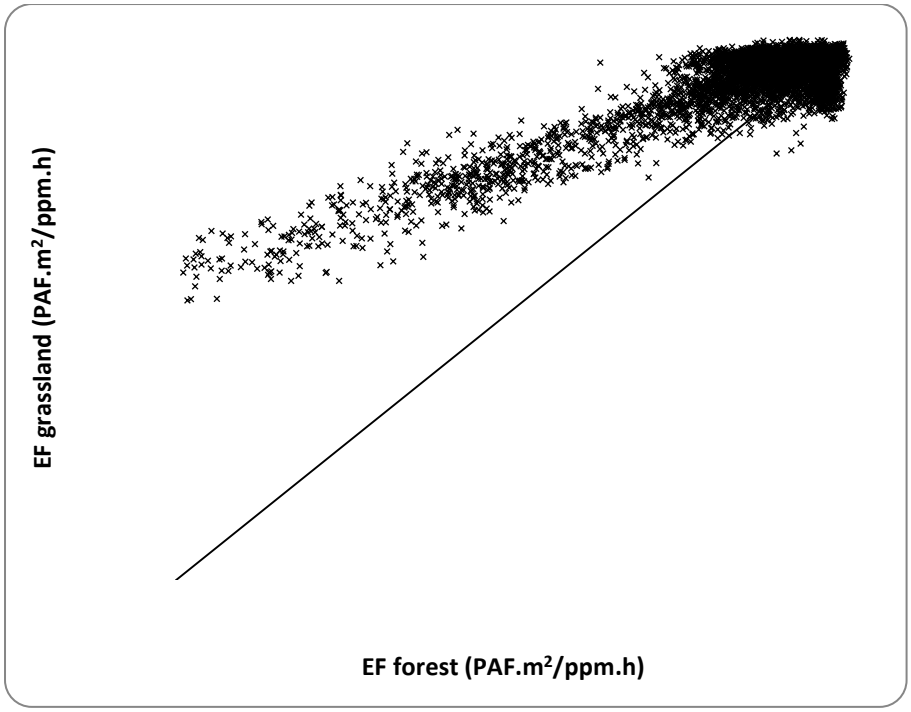

Figure S3.4. Comparison between effect factors for grasslands and forests in each grid.

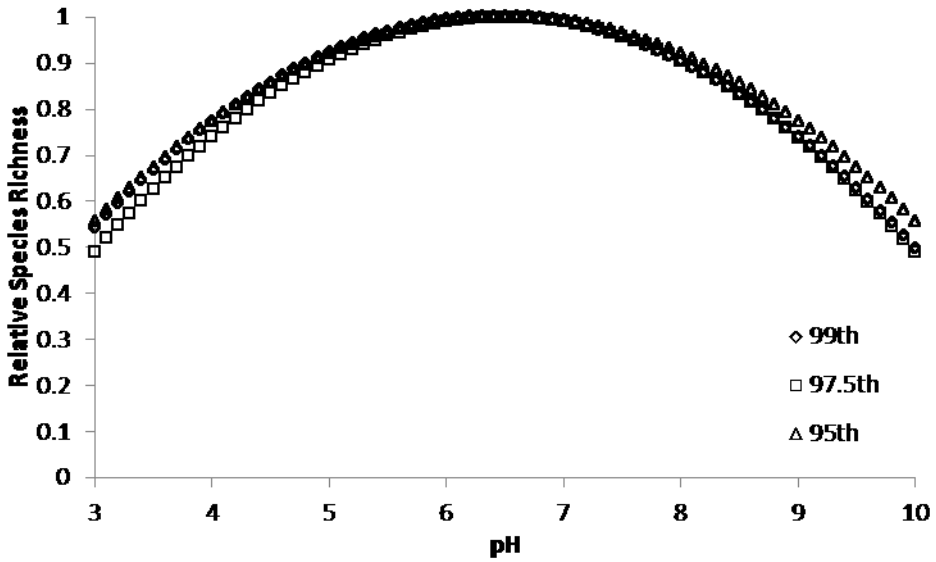

Figure S4.1. Field-based stressor response curves based on the quantile regression method derived with three quantiles, the 95th, 97.5th and 99th.

Table S4.1. The regression coefficients (with 95th percentile confidence intervals) for the regression models based on the 95th, 97.5th and 99th quantile.

Quantile	**95**	**97.5**	**99**
Intercept	-0.75 (-1.13 to -0.31)	-0.54 (-0.94 to -0.11)	-0.62 (-1.00 to -0.12)
pH	0.54 (0.32 to 0.74)	0.46 (0.28 to 0.65)	0.51 (0.32 to 0.73)
pH^2	-0.04 (-0.06 to -0.03)	-0.04 (-0.07 to -0.02)	-0.04 (-0.05 to -0.03)

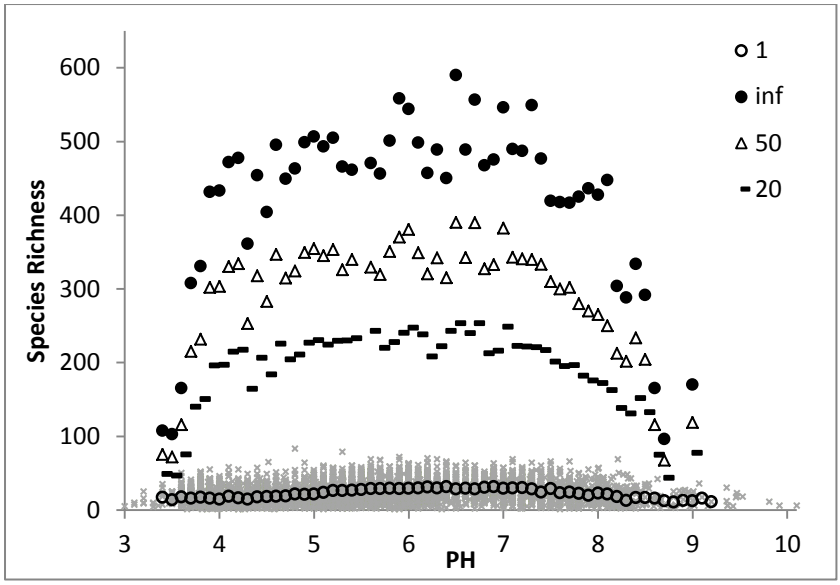

Figure S4.2. Field-based stressor response curves for pH based on the pooled sample method derived with infinite, 50, 20 and 1 samples. Observed species richness is plotted in gray.

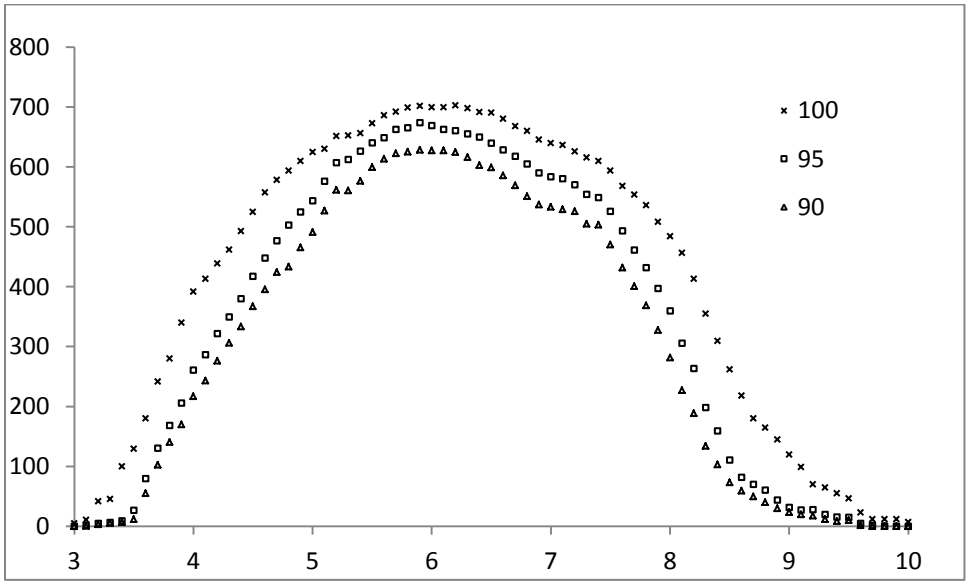

Figure S4.3. Field-based response curves for pH based on the occurrence range method derived with the 100th, 95th and 90th percentiles.

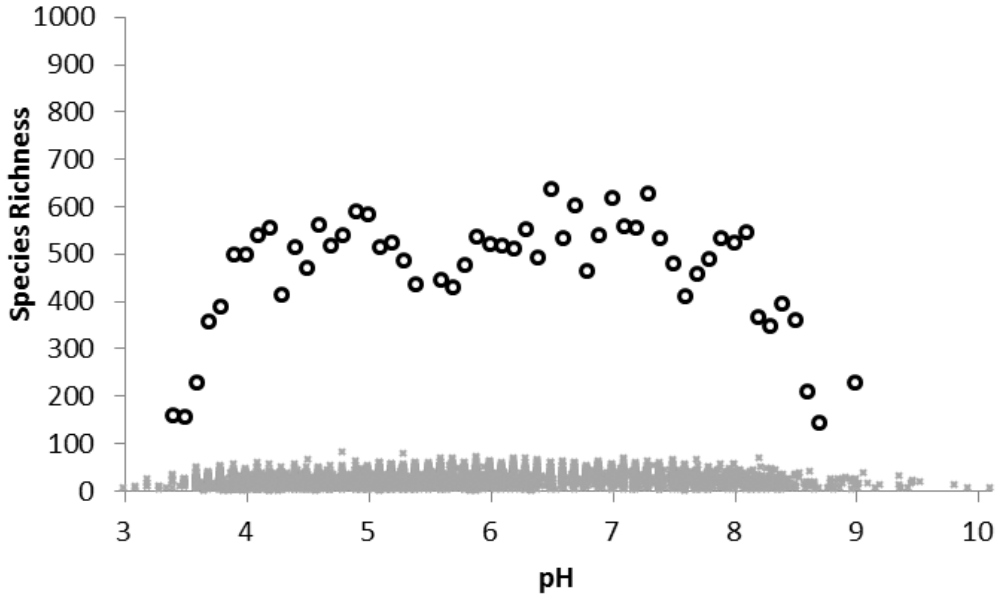

Figure S4.4. Field-based response curves for pH based on the pooled sample method derived for grasslands, forests and heathland. Observed SR is plotted in gray.

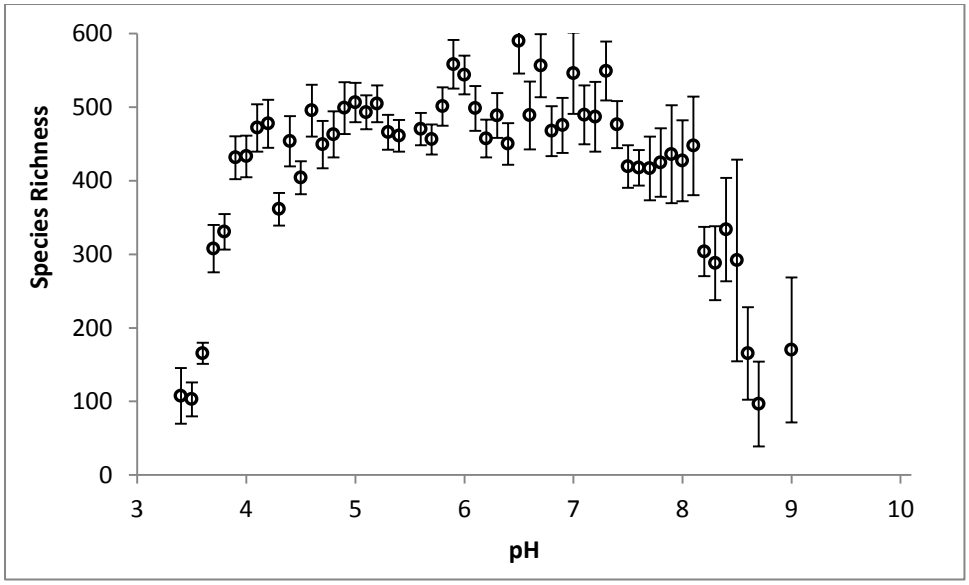

Figure S4.5. Field-based response curves for pH based on the pooled sample method derived with 5th and 95th confidence intervals.

Table S4.2. The number of relevés, average observed SR and estimated SR per interval are given for each method.

Occurrence range					Quantile regression					Pooled sample				
Min	Max	Number of relevés	Average observedSR	Estimated SR	Min	Max	Number of relevés	Average observedSR	Estimated SR	Min	Max	Number of relevés	Average observedSR	Estimated SR
3.0	3.1	2.0	7.3	2.0	3.0	3.1	2.0	7.3	33.4	3.4	3.5	17.0	16.9	155.4
3.1	3.2	4.0	15.0	6.0	3.1	3.2	4.0	15.0	33.6	3.5	3.6	54.0	20.8	151.8
3.2	3.3	3.0	7.7	8.0	3.2	3.3	3.0	7.7	33.9	3.6	3.7	75.0	20.1	222.4
3.3	3.4	7.0	19.9	12.0	3.3	3.4	7.0	19.9	34.2	3.7	3.8	111.0	22.6	344.7
3.4	3.5	17.0	16.9	34.0	3.4	3.5	17.0	16.9	34.6	3.8	3.9	128.0	19.4	374.7
3.5	3.6	54.0	20.8	111.0	3.5	3.6	54.0	20.8	35.0	3.9	4.0	146.0	17.9	482.3
3.6	3.7	75.0	20.1	165.0	3.6	3.7	75.0	20.1	35.5	4.0	4.1	118.0	17.4	481.2
3.7	3.8	111.0	22.6	213.0	3.7	3.8	111.0	22.6	36.0	4.1	4.2	121.0	16.1	520.9
3.8	3.9	128.0	19.4	270.0	3.8	3.9	128.0	19.4	36.5	4.2	4.3	112.0	15.0	534.5
3.9	4.0	146.0	17.9	339.0	3.9	4.0	146.0	17.9	37.0	4.3	4.4	108.0	17.4	398.3
4.0	4.1	118.0	17.4	372.0	4.0	4.1	118.0	17.4	37.6	4.4	4.5	195.0	17.9	497.9
4.1	4.2	121.0	16.1	418.0	4.1	4.2	121.0	16.1	38.1	4.5	4.6	120.0	18.8	455.2
4.2	4.3	112.0	15.0	454.0	4.2	4.3	112.0	15.0	38.7	4.6	4.7	107.0	17.7	543.5
4.3	4.4	108.0	17.4	494.0	4.3	4.4	108.0	17.4	39.3	4.7	4.8	102.0	20.8	498.3
4.4	4.5	195.0	17.9	554.0	4.4	4.5	195.0	17.9	40.0	4.8	4.9	108.0	19.5	520.0
4.5	4.6	120.0	18.8	582.0	4.5	4.6	120.0	18.8	40.6	4.9	5.0	164.0	19.7	568.8
4.6	4.7	107.0	17.7	619.0	4.6	4.7	107.0	17.7	41.2	5.0	5.1	182.0	22.0	563.3
4.7	4.8	102.0	20.8	653.0	4.7	4.8	102.0	20.8	41.9	5.1	5.2	187.0	24.8	495.1
4.8	4.9	108.0	19.5	682.0	4.8	4.9	108.0	19.5	42.5	5.2	5.3	163.0	24.7	506.7
4.9	5.0	164.0	19.7	725.0	4.9	5.0	164.0	19.7	43.1	5.3	5.4	192.0	26.3	467.9
5.0	5.1	182.0	22.0	768.0	5.0	5.1	182.0	22.0	43.8	5.4	5.5	214.0	25.1	421.3
5.1	5.2	187.0	24.8	810.0	5.1	5.2	187.0	24.8	44.4	5.5	5.6	226.0	27.0	426.7
5.2	5.3	163.0	24.7	817.0	5.2	5.3	163.0	24.7	45.0	5.6	5.7	238.0	28.1	430.5
5.3	5.4	192.0	26.3	835.0	5.3	5.4	192.0	26.3	45.6	5.7	5.8	204.0	27.5	416.1
5.4	5.5	214.0	25.1	854.0	5.4	5.5	214.0	25.1	46.2	5.8	5.9	165.0	26.8	461.0
5.5	5.6	226.0	27.0	865.0	5.5	5.6	226.0	27.0	46.8	5.9	6.0	168.0	28.1	518.4
5.6	5.7	238.0	28.1	884.0	5.6	5.7	238.0	28.1	47.3	6.0	6.1	158.0	26.7	503.7
5.7	5.8	204.0	27.5	887.0	5.7	5.8	204.0	27.5	47.8	6.1	6.2	148.0	30.1	500.5
5.8	5.9	165.0	26.8	899.0	5.8	5.9	165.0	26.8	48.3	6.2	6.3	120.0	26.7	494.2
5.9	6.0	168.0	28.1	892.0	5.9	6.0	168.0	28.1	48.8	6.3	6.4	103.0	30.4	532.6
6.0	6.1	158.0	26.7	884.0	6.0	6.1	158.0	26.7	49.2	6.4	6.5	92.0	27.9	475.1
6.1	6.2	148.0	30.1	881.0	6.1	6.2	148.0	30.1	49.6	6.5	6.6	65.0	29.1	615.1
6.2	6.3	120.0	26.7	874.0	6.2	6.3	120.0	26.7	50.0	6.6	6.7	75.0	27.6	513.9

6.3	6.4	103.0	30.4	867.0	6.3	6.4	103.0	30.4	50.3	6.7	6.8	74.0	28.8	581.6
6.4	6.5	92.0	27.9	853.0	6.4	6.5	92.0	27.9	50.5	6.8	6.9	71.0	33.0	447.5
6.5	6.6	65.0	29.1	838.0	6.5	6.6	65.0	29.1	50.8	6.9	7.0	85.0	29.3	519.3
6.6	6.7	75.0	27.6	824.0	6.6	6.7	75.0	27.6	50.9	7.0	7.1	78.0	28.3	597.2
6.7	6.8	74.0	28.8	795.0	6.7	6.8	74.0	28.8	51.1	7.1	7.2	68.0	28.3	537.6
6.8	6.9	71.0	33.0	787.0	6.8	6.9	71.0	33.0	51.1	7.2	7.3	90.0	26.9	535.9
6.9	7.0	85.0	29.3	780.0	6.9	7.0	85.0	29.3	51.2	7.3	7.4	157.0	19.4	606.2
7.0	7.1	78.0	28.3	754.0	7.0	7.1	78.0	28.3	51.1	7.4	7.5	74.0	28.6	513.4
7.1	7.2	68.0	28.3	741.0	7.1	7.2	68.0	28.3	51.0	7.5	7.6	71.0	21.7	463.4
7.2	7.3	90.0	26.9	720.0	7.2	7.3	90.0	26.9	50.8	7.6	7.7	55.0	23.9	397.6
7.3	7.4	157.0	19.4	713.0	7.3	7.4	157.0	19.4	50.6	7.7	7.8	61.0	21.0	441.6
7.4	7.5	74.0	28.6	683.0	7.4	7.5	74.0	28.6	50.3	7.8	7.9	43.0	22.7	473.7
7.5	7.6	71.0	21.7	641.0	7.5	7.6	71.0	21.7	49.9	7.9	8.0	54.0	23.1	515.1
7.6	7.7	55.0	23.9	599.0	7.6	7.7	55.0	23.9	49.4	8.0	8.1	43.0	21.6	506.3
7.7	7.8	61.0	21.0	561.0	7.7	7.8	61.0	21.0	48.9	8.1	8.2	50.0	15.6	526.6
7.8	7.9	43.0	22.7	516.0	7.8	7.9	43.0	22.7	48.2	8.2	8.3	45.0	16.9	354.7
7.9	8.0	54.0	23.1	467.0	7.9	8.0	54.0	23.1	47.5	8.3	8.4	27.0	21.3	336.1
8.0	8.1	43.0	21.6	387.0	8.0	8.1	43.0	21.6	46.7	8.4	8.5	13.0	20.1	382.7
8.1	8.2	50.0	15.6	334.0	8.1	8.2	50.0	15.6	45.8	8.5	8.6	12.0	18.3	348.6
8.2	8.3	45.0	16.9	265.0	8.2	8.3	45.0	16.9	44.8	8.6	8.7	10.0	14.6	202.1
8.3	8.4	27.0	21.3	202.0	8.3	8.4	27.0	21.3	43.8	8.7	8.8	5.0	14.2	140.4
8.4	8.5	13.0	20.1	140.0	8.4	8.5	13.0	20.1	42.6	8.8	8.9	7.0	14.6	
8.5	8.6	12.0	18.3	104.0	8.5	8.6	12.0	18.3	41.3	8.9	9.0	6.0	18.5	
8.6	8.7	10.0	14.6	89.0	8.6	8.7	10.0	14.6	39.9	9.0	9.1	6.0	18.5	221.0
8.7	8.8	5.0	14.2	74.0	8.7	8.8	5.0	14.2	38.4					
8.8	8.9	7.0	14.6	60.0	8.8	8.9	7.0	14.6	36.8					
8.9	9.0	6.0	18.5	45.0	8.9	9.0	6.0	18.5	35.1					
9.0	9.1	6.0	18.5	36.0	9.0	9.1	6.0	18.5	33.2					
9.1	9.2	2.0	8.0	33.0	9.1	9.2	2.0	8.0	31.3					
9.2	9.3	0.0			9.2	9.3	0.0		29.2					
9.3	9.4	4.0	13.5	22.0	9.3	9.4	4.0	13.5	27.0					
9.4	9.5	4.0	16.0	19.0	9.4	9.5	4.0	16.0	24.7					
9.5	9.6	1.0	18.0	6.0	9.5	9.6	1.0	18.0	22.3					
9.6	9.7	0.0			9.6	9.7	0.0		19.7					
9.7	9.8	0.0			9.7	9.8	0.0		16.9					
9.8	9.9	1.0	11.0	0.0	9.8	9.9	1.0	11.0	14.1					
9.9	10.0	1.0	6.0	0.0	9.9	10.0	1.0	6.0	11.1					
10.0	10.1	1.0	6.0	0.0	10.0	10.1	1.0	6.0	7.9					

Table S5.1. A description of the selected grassland and forest vegetation types. Numbers between brackets correspond to the vegetation classification of Schaminée et al. (1995)

Grasslands, hemming and dry heath
Moderate nutrient-rich grasslands (12)
Open grasslands (13,14)
Nutrient poor, closed grasslands (15)
Moderate nutrient-rich grasslands (16)
Hem (17,18)
Nutrient poor, closed grasslands (19)
Dry heath (20)
Brushwood, thicket and forests
Brushwood (32-34)
Thicket (35-37)
Wet forest (38-40)
Dry forest (41-43)

Table S5.2. Selected red list species per red list category, vulnerable species (VU), endangered species (EN) and critically endangered species (CR)

Species	**Category**
Actaea spicata	EN
Agrimonia procera	VU
Agrostemma githago	VU
Alchemilla filicaulis	EN
Alchemilla glabra	EN
Alchemilla monticola	CR
Allium oleraceum	VU
Alopecurus bulbosus	EN
Althaea officinalis	VU
Alyssum alyssoides	CR
Anacamptis morio	EN
Anagallis arvensis subsp. foemina	EN
Andromeda polifolia	VU
Antennaria dioica	CR
Anthemis arvensis	VU
Anthemis cotula	EN
Anthoxanthum aristatum	VU
Apium graveolens	VU
Apium inundatum	EN
Apium repens	CR
Arabis glabra	VU
Armeria maritima	VU
Arnica montana	EN
Arnoseris minima	EN
Artemisia absinthium	VU

Artemisia campestris subsp. campestris	CR
Artemisia campestris subsp. maritima	VU
Artemisia maritima	VU
Asparagus officinalis subsp. prostratus	VU
Asperugo procumbens	CR
Atriplex laciniata	EN
Atriplex pedunculata	CR
Atriplex portulacoides	VU
Atropa bella-donna	EN
Avena fatua	VU
Baldellia ranunculoides subsp. ranunculoides	EN
Baldellia ranunculoides subsp. repens	VU
Blysmus compressus	EN
Blysmus rufus	VU
Botrychium lunaria	EN
Briza media	VU
Bromopsis erecta	VU
Bromopsis ramosa subsp. benekenii	EN
Bromopsis ramosa subsp. ramosa	CR
Bromus racemosus	VU
Bromus secalinus	EN
Bunium bulbocastanum	VU
Bupleurum tenuissimum	EN
Calamagrostis stricta	EN
Callitriche hermaphroditica	EN
Campanula glomerata	CR
Campanula rapunculus	VU
Carduus tenuiflorus	VU
Carex appropinquata	VU
Carex aquatilis	VU
Carex buxbaumii	VU
Carex caryophyllea	VU
Carex cespitosa	VU
Carex diandra	EN
Carex digitata	VU
Carex dioica	CR
Carex ericetorum	VU
Carex flava	VU
Carex hostiana	EN
Carex lasiocarpa	VU
Carex pulicaris	EN
Carlina vulgaris	EN
Carum carvi	EN
Carum verticillatum	CR
Centaurea calcitrapa	CR
Centunculus minimus	EN

Cephalanthera damasonium	EN
Cephalanthera longifolia	VU
Cephalanthera rubra	EN
Cerastium fontanum subsp. holosteoides	VU
Chenopodium bonus-henricus	CR
Chenopodium murale	EN
Chenopodium vulvaria	EN
Chondrilla juncea	VU
Cicendia filiformis	EN
Cicuta virosa	VU
Cirsium acaule	VU
Cirsium dissectum	VU
Cladium mariscus	VU
Clematis viticella	EN
Clinopodium acinos	EN
Cochlearia officinalis subsp. anglica	VU
Cochlearia officinalis subsp. officinalis	EN
Colchicum autumnale	VU
Consolida regalis	CR
Cornus suecica	EN
Corrigiola litoralis	EN
Crataegus laevigata	VU
Crepis vesicaria subsp. taraxacifolia	VU
Cruciata laevipes	VU
Cuscuta epithymum	VU
Cystopteris fragilis	EN
Dactylorhiza incarnata	VU
Dactylorhiza majalis subsp. Majalis	VU
Dactylorhiza majalis subsp. sphagnicola	CR
Dactylorhiza viridis	CR
Deschampsia setacea	CR
Dianthus armeria	VU
Dianthus carthusianorum	EN
Dianthus deltoides	VU
Drosera anglica	CR
Elatine hydropiper	EN
Eleocharis quinqueflora	EN
Eleogiton fluitans	VU
Epilobium obscurum	VU
Epipactis atrorubens	CR
Epipactis palustris	VU
Equisetum sylvaticum	VU
Equisetum variegatum	VU
Erigeron acer	VU
Eriophorum gracile	CR
Eriophorum latifolium	CR

Eriophorum vaginatum	VU
Erodium lebelii	EN
Erucastrum gallicum	VU
Eryngium maritimum	VU
Erysimum cheiri	CR
Erysimum virgatum	CR
Euphorbia exigua	VU
Euphorbia palustris	VU
Euphorbia platyphyllos	VU
Euphorbia seguieriana	CR
Euphorbia stricta	VU
Euphrasia officinalis	CR
Festuca ovina subsp. guestphalica	CR
Filago arvensis	VU
Filago lutescens	CR
Filipendula vulgaris	VU
Fragaria moschata	CR
Fritillaria meleagris	EN
Galeopsis angustifolia	CR
Galeopsis ladanum	CR
Galeopsis segetum	VU
Galeopsis speciosa	VU
Galium boreale	EN
Galium pumilum	VU
Genista germanica	CR
Genista pilosa	VU
Genista tinctoria	EN
Gentianella amarella	EN
Gentianella campestris	EN
Gentianella germanica	EN
Gentianopsis ciliata	EN
Geranium columbinum	VU
Geum rivale	VU
Glaux maritima	VU
Gratiola officinalis	EN
Gymnadenia conopsea	CR
Hammarbya paludosa	CR
Helictotrichon pratense	VU
Helleborus viridis	EN
Herminium monorchis	CR
Hieracium lactucella	CR
Hieracium murorum	VU
Hierochloe odorata	VU
Holosteum umbellatum	EN
Honckenya peploides	VU
Hordeum marinum	EN

Huperzia selago	VU
Hyoscyamus niger	EN
Hypericum elodes	VU
Hypericum hirsutum	VU
Hypericum maculatum subsp. maculatum	EN
Hypericum montanum	EN
Hypericum pulchrum	EN
Hypochaeris glabra	EN
Illecebrum verticillatum	VU
Inula britannica	VU
Isoetes echinospora	EN
Isoetes lacustris	EN
Jacobaea paludosa	VU
Juncus capitatus	CR
Juncus maritimus	VU
Juncus pygmaeus	EN
Juncus tenageia	EN
Kickxia elatine	VU
Kickxia spuria	EN
Knautia arvensis	VU
Koeleria pyramidata	VU
Lathraea squamaria	EN
Lathyrus aphaca	CR
Lathyrus linifolius	CR
Lathyrus palustris	VU
Leersia oryzoides	VU
Legousia hybrida	CR
Legousia speculum-veneris	EN
Leontodon hispidus	VU
Lepidium campestre	EN
Leucojum aestivum	VU
Lilium bulbiferum subsp. croceum	CR
Linnaea borealis	EN
Linum catharticum	VU
Liparis loeselii	EN
Lithospermum arvense	EN
Littorella uniflora	VU
Lobelia dortmanna	CR
Ludwigia palustris	EN
Luronium natans	VU
Lycopodium clavatum	EN
Lycopodium tristachyum	CR
Malva pusilla	VU
Marrubium vulgare	CR
Medicago falcata	VU
Melampyrum arvense	CR

Mentha pulegium	VU
Mentha suaveolens	VU
Minuartia hybrida	CR
Misopates orontium	VU
Monotropa hypopitys	EN
Morella caroliniensis	VU
Myosotis scorpioides subsp. nemorosa	CR
Myosotis stricta	VU
Myriophyllum alterniflorum	VU
Najas minor	EN
Narthecium ossifragum	VU
Neottia nidus-avis	CR
Nepeta cataria	VU
Odontites vernus subsp. vernus	EN
Oenanthe lachenalii	EN
Oenanthe silaifolia	CR
Ophrys insectifera	CR
Orchis anthropophora	EN
Orchis mascula	EN
Orchis purpurea	CR
Orobanche minor	VU
Orobanche purpurea	VU
Orobanche rapum-genistae	EN
Parapholis strigosa	VU
Parnassia palustris	VU
Pedicularis palustris	VU
Pedicularis sylvatica	VU
Petrorhagia prolifera	VU
Peucedanum carvifolia	VU
Phyteuma spicatum subsp. nigrum	EN
Phyteuma spicatum subsp. spicatum	EN
Pimpinella saxifraga	VU
Pinguicula vulgaris	EN
Plantago maritima	VU
Plantago media	VU
Platanthera bifolia	EN
Platanthera montana	VU
Poa chaixii	EN
Polygala comosa	VU
Polygala serpyllifolia	VU
Polygala vulgaris	VU
Potamogeton acutifolius	VU
Potamogeton alpinus	EN
Potamogeton compressus	VU
Potamogeton gramineus	VU
Potamogeton obtusifolius	VU

Potamogeton praelongus	EN
Potentilla sterilis	VU
Potentilla tabernaemontani	EN
Primula veris	VU
Primula vulgaris	VU
Puccinellia distans subsp. borealis	VU
Puccinellia fasciculata	VU
Puccinellia maritima	VU
Pyrola minor	EN
Pyrola rotundifolia	VU
Radiola linoides	EN
Ranunculus arvensis	CR
Ranunculus auricomus	VU
Ranunculus baudotii	VU
Ranunculus fluitans	EN
Ranunculus hederaceus	VU
Ranunculus ololeucos	EN
Ranunculus polyanthemos	CR
Rhynchospora alba	VU
Rosa agrestis	CR
Rosa dumalis	VU
Rosa micrantha	VU
Rosa tomentosa	EN
Rosa villosa	EN
Rubus saxatilis	CR
Ruppia cirrhosa	EN
Ruppia maritima	VU
Sagina nodosa	VU
Salicornia europaea	VU
Salsola tragus	VU
Salvia pratensis	VU
Salvia verbenaca	VU
Salvia verticillata	CR
Sambucus ebulus	VU
Sanicula europaea	VU
Saxifraga granulata	EN
Scabiosa columbaria	EN
Scandix pecten-veneris	CR
Scheuchzeria palustris	CR
Schoenoplectus pungens	EN
Schoenoplectus triqueter	EN
Schoenus nigricans	EN
Scleranthus perennis	CR
Scorzonera humilis	EN
Scutellaria minor	EN
Sedum rupestre	VU

Sedum sexangulare	VU
Selinum carvifolia	CR
Sherardia arvensis	VU
Silaum silaus	VU
Silene baccifera	EN
Silene gallica	EN
Silene noctiflora	EN
Sisymbrium loeselii	EN
Solidago virgaurea	VU
Sparganium angustifolium	VU
Sparganium natans	EN
Spiranthes spiralis	CR
Stachys arvensis	VU
Stachys officinalis	EN
Stachys recta	EN
Suaeda maritima	VU
Teucrium botrys	CR
Teucrium montanum	CR
Teucrium scordium	CR
Thesium humifusum	CR
Thlaspi perfoliatum	VU
Thymus serpyllum	EN
Torilis arvensis	EN
Torilis nodosa	VU
Tragopogon pratensis subsp. orientalis	VU
Trichophorum cespitosum subsp. germanicum	VU
Trifolium medium	VU
Trifolium micranthum	EN
Trifolium striatum	VU
Trifolium subterraneum	EN
Triglochin maritima	VU
Tuberaria guttata	CR
Tulipa sylvestris	VU
Utricularia intermedia	EN
Utricularia minor	VU
Vaccinium uliginosum	VU
Valeriana dioica	VU
Valerianella carinata	VU
Valerianella dentata	CR
Valerianella rimosa	CR
Veronica opaca	VU
Veronica polita	VU
Veronica praecox	EN
Veronica prostrata	CR
Veronica triphyllos	CR
Veronica verna	CR

Vicia lathyroides	VU
Vicia tenuifolia	VU
Vicia tetrasperma subsp. gracilis	VU
Vincetoxicum hirundinaria	VU
Viola hirta	VU
Viola lutea subsp. calaminaria	EN
Viola persicifolia	EN
Wahlenbergia hederacea	EN
Zostera marina	EN
Zostera noltei	CR

Table S5.3. Regression coefficients (including 95% confidence interval) for each quantile regression model.

	Intercept	**NO3**	**NO3^2**	**pH**	**pH^2**	**NO3:pH**
NO_3						
Grassland	45.9 (43.9-47.8)	0.8 (0.7-1.0)	-2.7 (-4- -1.7)	-	-	-
Forest	42.4 (41.3-43.5)	-1.4 (-2.0- -1.1)	-1.7 (-3.0- -1.0)	-	-	-
Grassland red list	4.2 (4.0-4.3)	0.5 (0.4-0.6)	1.2 (-1.4- -1.0)	-	-	-
Forest red list	4.2 (4.1-4.3)	-0.3 (-0.4- -0.2)	-0.6 (-1.0- -0.5)	-	-	-
NO_3 and pH						
Grassland	41.3 (39.9-42.8)	0.1 (0-0.2)	-1.0 (-1.2- -0.7)	4.7 (4.3-4.9)	-2.5 (-2.8- -2.1)	-0.9 (-1.2- -0.7)
Forest	40.9 (38.4-42.1)	-1.3 (-1.4- -1.2)	-0.9 (-1.0- -0.8)	3.8 (3.4-4.1)	-3.3 (-3.6- -3.0)	-1.5 (-1.6- -1.3)
Grassland red list	4.7 (4.5-5.0)	0.0 (-0.1-0.2)	-0.9 (-1.1 -0.7)	-0.2 (-0.3- -0.1)	-0.2 (-0.3- -0.1)	-0.7 (-0.9- -0.5)
Forest red list	4.6 (4.3-4.9)	-0.2 (-0.4-0)	-0.6 (-0.9- -0.4)	-0.2 (-0.3- -0.2)	-0.3 (-0.4- -0.1)	-0.6 (-0.6- -0.5)

Table S6.1. Regression coefficients and Bayesian Information Criterion (BIC) values for each constructed quantile regression model based on the diversity indices. The Gaussian model always had the lowest BIC. SR = species richness; H = Shannon index, He = species evenness, FRic = functional richness, FEve = functional evenness, FDiv = functional divergence.

		β_0	β_1	β_2	BIC
SR	**Gaussian**	-12.78	4.59	-0.36	4279.31*
	Linear	-0.17	0.26		4423.39
	Intercept	1.32			4432.24
H	**Gaussian**	-7.92	2.99	-0.24	3444.31*
	Linear	0.76	0.06		3571.45
	Intercept	1.11			3574.90
He	**Gaussian**	-2.02	1.17	-0.11	3617.11*
	Linear	2.14	-0.20		3630.70
	Intercept	0.95			3684.00
cFRic	**Gaussian**	10.19	-3.02	0.23	4787.21*
	Linear	2.07	-0.20		4848.93
	Intercept	0.91			4878.97
cFEve	**Gaussian**	3.77	-0.89	0.08	4161.99*
	Linear	1.06	0.04		4181.01
	Intercept	1.28			4180.22
cFDiv	**Gaussian**	8.32	-2.37	0.19	4117.84*
	Linear	1.70	-0.09		4206.11
	Intercept	1.22			4212.60
wFRic	**Gaussian**	10.16	-3.34	0.27	4178.04*
	Linear	0.02	-0.03		4247.10
	Intercept	-0.16			4257.59
wFEve	**Gaussian**	0.22	0.28	-0.02	3909.10*
	Linear	0.90	0.05		3919.33
	Intercept	1.19			3922.70
wFDiv	**Gaussian**	6.72	-2.21	0.18	3444.61*
	Linear	-0.03	-0.01		3534.96
	Intercept	-0.12			3538.00
hFRic	**Gaussian**	30.15	-9.27	0.70	4390.06*
	Linear	6.30	-0.84		5059.76
	Intercept	0.27			5433.24
hFEve	**Gaussian**	2.04	-0.40	0.04	3735.54*
	Linear	0.71	0.07		3748.76
	Intercept	1.11			3752.09
hFDiv	**Gaussian**	15.89	-5.04	0.40	3951.77*
	Linear	1.47	-0.21		4235.92
	Intercept	0.14			4362.66
sFRic	**Gaussian**	14.81	-4.36	0.34	4520.64*
	Linear	1.79	-0.09		4628.76
	Intercept	1.31			4630.68

sFEve	**Gaussian**	7.62	-1.98	0.15	4157.64*
	Linear	2.43	-0.21		4190.49
	Intercept	1.29			4227.80
sFDiv	**Gaussian**	0.40	0.35	-0.04	4608.38*
	Linear	2.01	-0.18		4618.41
	Intercept	1.06			4618.57
wCWM	**Gaussian**	8.39	-2.75	0.22	3216.70*
	Linear	-0.02	-0.01		3319.36
	Intercept	-0.08			3319.22
hCWM	**Gaussian**	13.87	-4.42	0.35	3994.29*
	Linear	1.16	-0.13		4224.20
	Intercept	0.34			4265.00
sCWM	**Gaussian**	-1.27	0.68	-0.05	3336.29*
	Linear	0.90	-0.01		3346.90
	Intercept	0.85			3345.25

Table S6.2. The 15 most frequent plant species in the releves between the 85th and 95th confidence interval of the functional richness response curve at three pH interval.

	FRic	
	Species	**Family**
pH 3.3-4.4	*Calluna vulgaris*	Ericaceae
	Carex pilulifera	Cyperacea
	Pinus sylvestris	Pinaceae
	Rumex acetosella	Polygonaceae
	Molinia caerulea	Poaceae
	Erica tetralix	Ericaceae
	Agrostis canina	Poaceae
	Galium saxatile	Rubiaceae
	Deschampsia flexuosa	Poaceae
	Potentilla erecta	Rosaceae
	Rhamnus frangula	Rhamnaceae
	Festuca ovina	Poaceae
	Genista pilosa	Fabaceae
	Juncus squarrosus	Juncaceae
	Festuca ovina	Poaceae
pH 5.9-6.9	*Anthoxanthum odoratum*	Poaceae
	Holcus lanatus	Poaceae
	Poa pratensis	Poaceae
	Festuca rubra	Poaceae
	Trifolium repens	Leguminosae
	Cardamine pratensis	Brassicaceae
	Ranunculus repens	Ranunculaceae
	Poa trivialis	Poaceae
	Agrostis stolonifera	Poaceae

	Agrostis species	Poaceae
	Carex nigra	Cyperacea
	Ranunculus acris	Ranunculaceae
	Taraxacum officinalis	Asteraceae
	Galium palustre	Rubiaceae
	Plantago lanceolata	Plantaginaceae
pH 7.8-8.8	*Poa pratensis*	Poaceae
	Dactylis glomerata	Poaceae
	Festuca rubra	Poaceae
	Elymus repens	Poaceae
	Agrostis stolonifera	Poaceae
	Poa trivialis	Poaceae
	Agrostis species	Poaceae
	Lolium perenne	Poaceae
	Holcus lanatus	Poaceae
	Plantago lanceolata	Plantaginaceae
	Achillea millefolium	Asteraceae
	Taraxacum species	Asteraceae
	Arrhenatherum elatius	Poaceae
	Glechoma hederacea	Lamiaceae
	Trisetum flavescens	poaceae

Table S6.3. The 15 most abundant plant species in the releves between the 85th and 95th confidence interval of the functional evenness response curve at three pH interval.

	Feve	
	Species	**Family**
pH 3.3-4.4	*Calluna vulgaris*	Ericaceae
	Deschampsia flexuosa	Poaceae
	Agrostis canina	Poaceae
	Festuca ovina	Poaceae
	Agrostis capillaris	Poaceae
	Molinia caerulea	Poaceae
	Carex nigra	Cyperacea
	Erica tetralix	Ericaceae
	Arnica montana	asteraceae
	Juniperus communis	Cupressaceae
	Nardus stricta	Poaceae
	Carex pilulifera	Cyperacea
	Galium saxatile	Rubiaceae
	Potentilla erecta	Rosaceae
	Holcus lanatus	Poaceae
pH 5.3-6.3	*Trifolium repens*	Leguminosae
	Agrostis capillaris	Poaceae
	Poa trivialis	Poaceae
	Agrostis species	Poaceae

	Lolium perenne	Poaceae
	Holcus lanatus	Poaceae
	Poa pratensis	Poaceae
	Agrostis stolonifera	Poaceae
	Ranunculus repens	Ranunculaceae
	Anthoxanthum odoratum	Poaceae
	Agrostis canina	Poaceae
	Festuca rubra	Poaceae
	Glyceria fluitans	Poaceae
	Cirsium dissectum	Asteraceae
	Erica tetralix	Ericaceae
pH 7.8-8.8	*Agrostis stolonifera*	Poaceae
	Elymus repens	Poaceae
	Trifolium repens	Leguminosae
	Lolium perenne	Poaceae
	Poa trivialis	Poaceae
	Festuca rubra	Poaceae
	Dactylis glomerata	Poaceae
	Festuca arundinacea	Poaceae
	Agrostis species	Poaceae
	Poa pratensis	Poaceae
	Juncus gerardi	Juncaceae
	Leontodon autumnalis	Asteraceae
	Corylus avellana	Betulaceae
	Holcus lanatus	Poaceae
	Alopecurus geniculatus	Poaceae

Table S6.4. The 15 most abundant plant species in the releves between the 85th and 95th confidence interval of the functional divergence response curve at three pH interval.

	Fdiv	
	Species	**Family**
pH 3.3-4.4	*Deschampsia flexuosa*	Poaceae
	Calluna vulgaris	Ericaceae
	Agrostis stolonifera	Poaceae
	Molinia caerulea	Poaceae
	Agrostis canina	Poaceae
	Erica tetralix	Ericaceae
	Arnica montana	asteraceae
	Agrostis species	Poaceae
	Festuca rubra	Poaceae
	Potentilla anserina	Rosaceae
	Nardus stricta	Poaceae
	Empetrum nigrum	Ericaceae
	Potentilla erecta	Rosaceae
	Galium saxatile	Rubiaceae

	Carex nigra	Cyperacea
pH 5.7-6.7	*Poa trivialis*	Poaceae
	Agrostis species	Poaceae
	Agrostis stolonifera	Poaceae
	Festuca rubra	Poaceae
	Holcus lanatus	Poaceae
	Trifolium repens	Leguminosae
	Lolium perenne	Poaceae
	Poa pratensis	Poaceae
	Anthoxanthum odoratum	Poaceae
	Ranunculus acris	Ranunculaceae
	Ranunculus repens	Ranunculaceae
	Rumex acetosa	Polygonaceae
	Festuca pratensis	Poaceae
	Plantago lanceolata	Plantaginaceae
	Cardamine pratensis	Brassicaceae
pH 7.8-8.8	*Elymus repens*	Poaceae
	Agrostis stolonifera	Poaceae
	Agrostis species	Poaceae
	Festuca rubra	Poaceae
	Poa trivialis	Poaceae
	Elymus athericus	Poaceae
	Dactylis glomerata	Poaceae
	Trifolium repens	Leguminosae
	Poa pratensis	Poaceae
	Holcus lanatus	Poaceae
	Lolium perenne	Poaceae
	Potentilla anserina	Rosaceae
	Alopecurus pratensis	Poaceae
	Juncus gerardi	Juncaceae
	Corylus avellana	Betulaceae

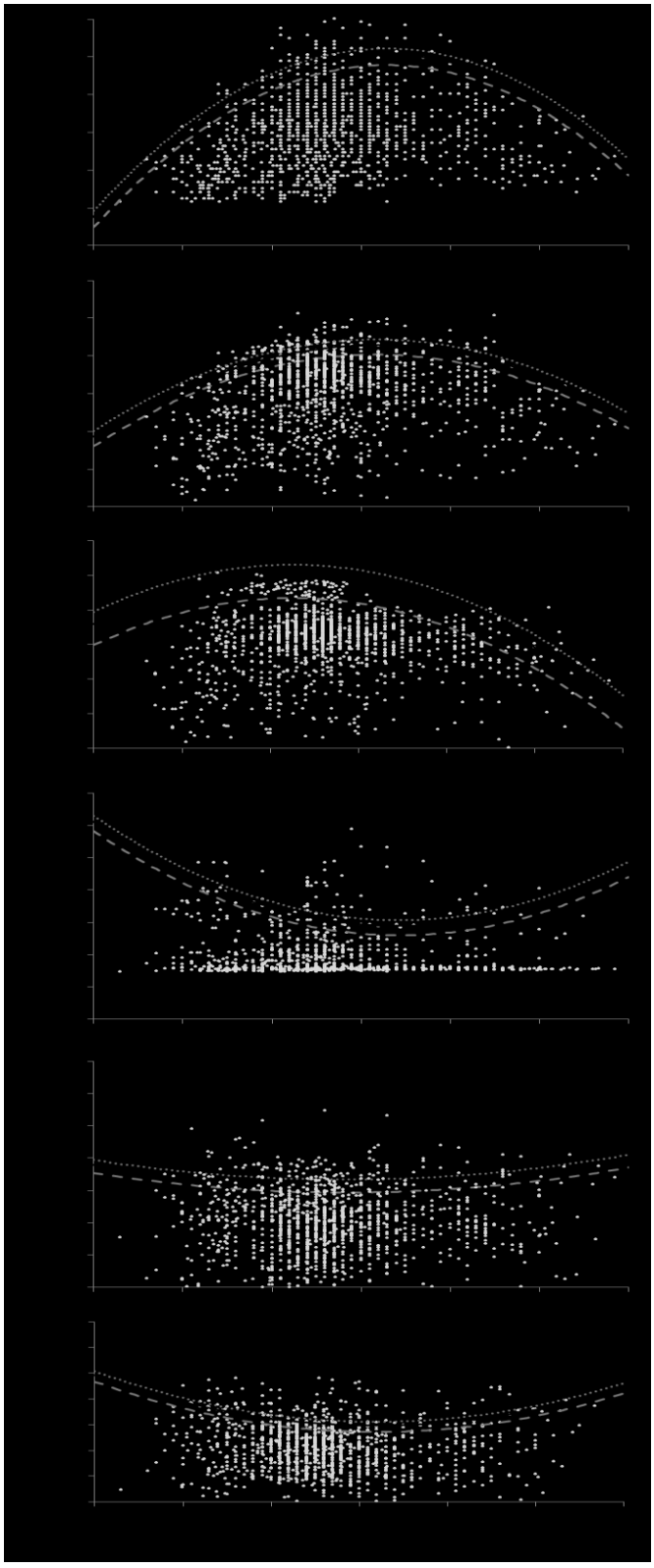

Figure S6.1. Response curves based on the 95th, 90th and 75th quantiles for taxonomic richness, evenness and diversity and functional richness, evenness and divergence.

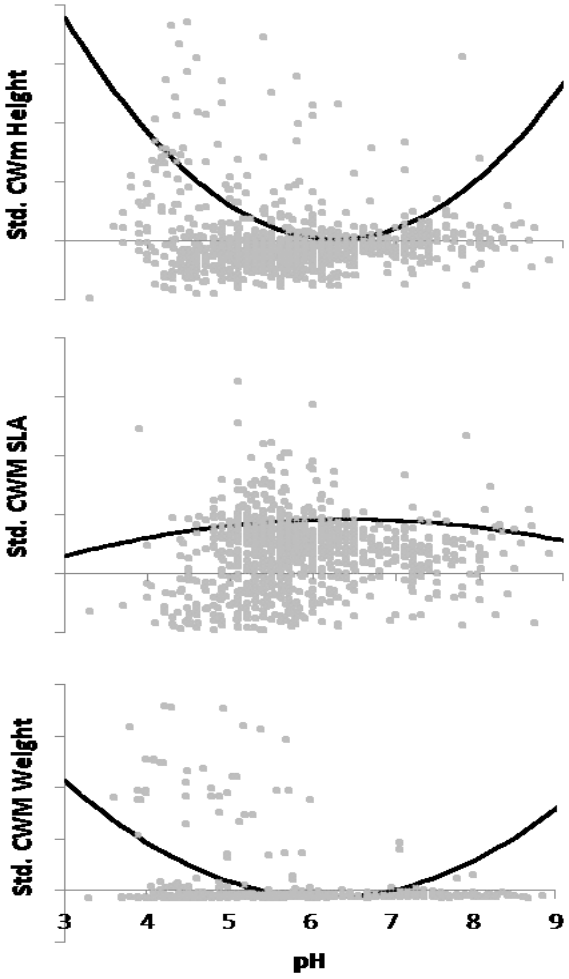

Figure S6.2. pH response curves for standardized community weighted means (CWM) for plant maximum height, specific leaf area (SLA) and average seed weight. The y-axis has steps of 1 standard deviation above or below the mean. The CWM of each individual trait was calculated as an indicator of the functional composition of the selected relevés . The CWM was calculated as the sum across all species products of each species trait value and their relative abundance (Garnier et al., 2004).

Text section S6.1: Equations

Species richness

Species richness (SR) was calculated for each relevé j as

$$SR_j = \sum_j s \tag{S1}$$

where s is a single species in relevé j.

Shannon index

Species diversity was calculated using Shannon diversity index (H), as it takes into account the evenness and richness of species present in the community (Tuomisto, 2010). Species diversity (H) was derived for each relevé j as

$$H_j = -\sum_{i=1}^{S} p_i \cdot \ln p_i \tag{S2}$$

where *S* is the total number of species in relevé j and p_i is the proportion of *S* made up of the *i*th species in relevé j.

Species evenness

Species evenness was calculated with the Shannon's equitability diversity index (E_H). E_H can be calculated for each relevé j by dividing H_j by $H_{j,MAX}$ (here $H_{max} = \ln S$). Equitability assumes a value between 0 and 1 with 1 being complete evenness.

$$E_{Hj} = H_j / H_{j,MAX} = H_j / \ln S \tag{S3}$$

Functional richness

The convex hull volume is used as a measure of the functional space occupied by a community. The convex hull is actually the minimum convex hull which includes all the species considered; the convex hull volume is then the volume inside this hull.

Thus, if two species a and b are inside the convex hull volume, whose coordinates (i.e., traits values) are respectively (xa1, xa2, … xaT) and (xb1, xb2, … xbT), then any hypothetical species with coordinates (Kxa1 + (1 − K)xb1, Kxa2 + (1 − K)xb2, …, KxaT + (1 − K)xbT) for 0 ≤ K ≤ 1 is also in the convex hull volume. This measure of space occupancy corresponds to a multivariate range. Any species whose trait values are less extreme for all traits than those of the two existing species will be included inside the convex hull volume. Basically, the convex hull volume algorithm determines the most extreme points, links them to build the convex hull, and finally calculates the volume inside.

The convex hull volume is computed with the Quickhull algorithm (Barber et al. 1996). The number of species must be higher than the number of traits (S > T), and the species must not be distributed in a line (in which case the hull volume is zero). The program returns the volume and the identity of the species forming the vertices.

Functional evenness

Functional evenness (Villeger et al., 2008) was derived as:

$$FEve = \frac{\sum_{l=1}^{S-1} \min(PWE_l \frac{1}{S-1}) - \frac{1}{S-1}}{1 - \frac{1}{S-1}} \quad \text{(S4)}$$

where S is the number of species, l is the branch length. PWE_l is a partial weighted evenness:

$$PWE_l = \frac{EW_l}{\sum_{l=l}^{S-1} EW_l} \quad \text{(S5)}$$

where EW_l is the weighted evenness:

$$EW_l = \frac{dist(i,j)}{w_i + w_j} \quad \text{(S6)}$$

where dist (i,j) is the Euclidean distance between species i and j, the species involved is branch l, and w_i and w_j are the relative abundance of these species. **Functional divergence**

Firstly, the coordinates of the center of gravity GV (g1, g2, … gT) of the V species forming the vertices of the convex hull are calculated as follows:

$$g_k = \frac{1}{V} \sum_{i=1}^{V} x_{ik} \quad \text{(S7)}$$

where x_{ik} is the coordinate of species i on trait k [1, T].

Second, for each of the S species, we calculate the Euclidean distance to this center of gravity:

$$dG_i = \sqrt{\sum_{k=1}^{T} (x_{ik} - g_k)^2}. \quad \text{(S8)}$$

The mean distance of the S species to the center of gravity $\overline{dG}$ is then calculated:

$$\overline{dG} = \frac{1}{S} \sum_{i=1}^{S} dG_i. \quad \text{(S9)}$$

Then, the sum of abundance-weighted deviances (Δd) and absolute abundance-weighted deviances (Δ|d|) for distances from the center of gravity are calculated across the species:

$$\Delta d = \sum_{i=1}^{S} w_i \times (dG_i - \overline{dG}) \quad \text{(S10)}$$

And

$$\Delta |d| = \sum_{i=1}^{S} w_i \times |dG_i - \overline{dG}|. \quad \text{(S11)}$$

Functional divergence may then be calculated as

$$\mathrm{FDiv} = \frac{\Delta d + \overline{dG}}{\Delta |d| + \overline{dG}}. \quad \text{(S12)}$$

Text section S6.2: Species composition

Species composition along the pH gradient was determined to better understand the responses of the functional diversity indices. Three intervals of one pH unit were selected, representing low (3.3-4.3), median (half a pH unit above and below the minimum pH of the respective response curve) and high (7.8-8.8) pH values. Within each pH interval the species composition of the releves within the 95th confidence interval of the response curve were taken into account. For functional richness the 15 most common species at each pH interval were considered, for functional evenness and divergence the 15 most abundant species taken.

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Summary

The species sensitivity distribution (SSD) approach has proven useful to quantify the relationships between species and specific environmental stressors. SSDs based on laboratory experiments, however, have several limitations. First, they are generally derived from tests with a few easily cultured aquatic species that may be absent from the field site of concern. Second, SSDs for non-toxic stressors in the terrestrial environment are mostly not available. Third, endpoints on the community level, for instance reflecting taxonomic or functional diversity, are typically lacking. To address these limitations, this thesis aimed to develop and apply SSDs to quantify the effects of ozone formation, acidification and eutrophication on taxonomic and functional characteristics of terrestrial plant communities. More specifically, the SSDs developed aimed to (1) cover a representative species pool, (2) include more environmental factors, and (3) apply novel, assemblage-level endpoints (**chapter 1**). First, laboratory-based SSDs were developed and applied to set environmental quality standards (EQS) and to assess environmental risk of tropospheric ozone for terrestrial plant communities (chapter 2, 3). Next, field-based SSDs (f-SSDs) were developed and applied to set EQS for acidification (chapter 4) and eutrophication (chapter 5) and to understand biodiversity patterns along a pH gradient by addressing functional endpoints (chapter 6).

Chapter 2 presented SSDs for ozone exposure of natural vegetation. SSDs were constructed for three species groups, i.e. trees, annual grassland species and perennial grassland species, using species-specific exposure-response laboratory data. The concentrations at which 10% and 50% biomass loss occurs (EC_{10} and EC_{50} values) were derived with linear exposure-response relationships and subsequently used to construct the SSDs. The SSDs were applied in two ways. First, EQS were calculated for each species group and compared to current critical levels for ozone exposure. The EQS corresponds to the concentration at which 5% of the species are affected (based on EC_{10}). Second, spatially explicit estimates were made of the potentially affected fraction of plant species in Northwestern Europe, based on ambient ozone concentrations. The results showed that the SSD-based critical levels (i.e. EQS) were lower than the current critical levels for ozone exposure, with conventional critical levels for ozone corresponding with 8-20% affected plant species.

In **chapter** 3, the SSDs for tropospheric ozone were used to derive spatially explicit characterization factors (CFs) for tropospheric ozone damage on natural vegetation caused by anthropogenic nitrogen oxides (NOx) and non-methane volatile organic compound (NMVOC) emissions for 65 European regions. The CFs were defined as the

area-integrated increase in the potentially affected fraction (PAF) of trees and grassland species due to a change in emissions of NOx and NMVOCs. Each CF consisted of a fate factor, quantifying the relationship between the emission of precursor substances and ozone exposure, and an area-integrated effect factor, quantifying the relationship between ozone exposure and the damage to natural vegetation. The CFs for NOx were on average 3.5 times larger than the CFs for NMVOC. The CFs were largest in south European regions for both NOx and NMVOC. Both the fate factor and effect factor contributed equally to the spatial differences found in the CFs.

In **chapter 4**, a comparison was made between three methods recently proposed for deriving SSDs based on field data (f-SSDs), based on a common dataset comprising field observations of grassland vegetation and measured pH values collected from 4,412 sampling sites in the Netherlands. One of the methods estimated species richness close to the maximum species richness realized at the sites, whereas the other two provided estimates of the potential species richness along the pH gradient. The resulting f-SSDs suggest that potential species richness of grasslands is slightly more sensitive to acidification than realized plant species richness. However, differences in corresponding EQS for acidification were small compared to the influence of intrinsic spatial differences in natural soil pH on the EQS. This finding provides evidence that natural background values are more important to consider in the derivation of EQS for pH than differences between the three f-SSD approaches.

In **chapter 5**, a procedure was proposed to derive field-based EQS conditional on other environmental factors. To illustrate the procedure, a dataset was used with species richness observations of grasslands and forests and accompanying measurements of soil nitrate (NO_3) and acidity (pH) collected from 981 sampling sites in the Netherlands. Species richness was related to NO_3 and pH with quantile regression, allowing for interaction effects. The resulting regression models were used to quantify potential confounding effects of different pH background levels on EQS for soil NO_3, whereby the EQS was quantified as the NO_3 concentration protective for 95% of the species pool in respectively grasslands and forests. This was done for the entire species pool as well as for red list species specifically. The EQS for NO_3 varied between 1.8 and 64.6 mg/kg between pH levels of 4-9, with the most stringent EQS at high pH. The results further showed that red list species are twice as sensitive to an increase in NO_3 compared to the entire species pool. These findings indicate that both natural background pH conditions and red list species are important factors to consider in the derivation of EQS for NO_3.

In **chapter 6** taxonomic and functional diversity of plant communities in grasslands were quantified and compared in relation to soil pH. Based on the same dataset that was used in chapter 4, functional and taxonomic diversity of grassland vegetation were related to soil pH with quantile regression and compared to null models representing random community assembly. Along the pH gradient, opposing patterns of taxonomic and functional diversity were found. Taxonomic diversity was highest at intermediate pH levels, whilst functional diversity increased towards both ends of the gradient, thereby differing from the null model expectations. The opposing patterns of taxonomic and functional diversity are not in line with environmental filtering and niche divergence hypotheses alone, suggesting a role for niche convergence and facilitation in the communities studied. Further, the results indicate that taxonomic and functional richness provide complementary information about community assembly and that a simultaneous analysis of both may help reveal the prevalence of different assembly processes.

Chapter 7 provided an integrated description of the applicability of the SSDs developed in this thesis, by discussing the specific goals as formulated in chapter 1. It was concluded that f-SSDs provide a suitable approach to cover a representative species pool, include more environmental factors and to apply novel, assemblage-level endpoints. Lab-based SSDs might primarily be used for deriving environmental quality standards for new, toxic substances, whilst f-SSDs can principally be used in environmental risk assessment, LCIA and biogeographical research. Provided that suitable monitoring data are available, f-SSDs are preferred because they can more easily account for both background conditions and other confounding environmental factors, which can have considerable influence on EQS. Further, f-SSDs can more easily assess the sensitivity of specific species groups. Hence, the f-SSD approach enables to assess environmental risk for specific sets of conditions and species. Although the results of this thesis suggest that it is important to consider background conditions, confounding factors and specific species groups, it remains to be further investigated what level of detail is actually required in deriving and applying f-SSDs.

Samenvatting

Soortgevoeligheidsverdelingen (SSDs) zijn nuttig gebleken om de relaties tussen soorten en specifieke milieufactoren te kwantificeren. SSDs op basis van laboratoriumproeven hebben echter meerdere beperkingen. Ten eerste zijn ze over het algemeen gebaseerd op een paar gemakkelijk te kweken aquatische soorten die veelal afwezig zijn in veldsituaties. Ten tweede zijn SSDs voor niet-toxische stressoren in het terrestrische milieu meestal niet beschikbaar. Ten slotte ontbreken effectindicatoren (eindpunten) vaak op gemeenschapsniveau, bijvoorbeeld indicatoren die het effect op taxonomische of functionele diversiteit kwantificeren. In dit proefschrift spelen deze beperkingen van de SSD-methode een centrale rol. Het doel is het ontwikkelen en toepassen van SSDs om de effecten van ozonvorming, verzuring en eutrofiëring op taxonomische en functionele kenmerken van terrestrische plantengemeenschappen te kwantificeren. De specifieke doelen van dit proefschrift zijn gericht op het ontwikkelen van SSDs die (1) uitgaan van een representatieve soortenpoel, (2) betrekking hebben op meer milieufactoren, en (3) nieuwe eindpunten op gemeenschapsniveau hebben (**hoofdstuk 1**). Eerst zijn SSDs gebaseerd op laboratoriumdata ontwikkeld voor troposferische ozon. Deze SSDs zijn gebruikt voor het afleiden van milieunormen en het uitvoeren van een risicoanalyse van troposferische ozon op terrestrische plantengemeenschappen (hoofdstuk 2 en 3). Vervolgens zijn SSDs gebaseerd op velddata (f-SSDs) ontwikkeld en toegepast voor het afleiden van milieunormen voor verzuring (hoofdstuk 4) en eutrofiëring (hoofdstuk 5). Ten slotte zijn f-SSDs met eindpunten op basis van functionele diversiteit gebruikt om biodiversiteitspatronen langs een pH-gradiënt beter te begrijpen (hoofdstuk 6).

In **hoofdstuk 2** zijn SSDs ontwikkeld om het effect van troposferische ozon op terrestrische vegetatie te kwantificeren. Door gebruik te maken van data uit soort-specifieke blootstellingsexperimenten, zijn SSDs afgeleid voor drie soortgroepen, namelijk eenjarige graslandsoorten, meerjarige graslandsoorten en bomen. De ozonconcentraties waarbij 10% en 50% biomassaverlies optreedt (EC10 en EC50 waarden) zijn bepaald met lineaire blootstelling-effect relaties en vervolgens gebruikt om de SSDs af te leiden. De SSDs zijn op twee manieren toegepast. Eerst zijn milieunormen afgeleid voor elke soortgroep en vergeleken met huidige normen voor blootstelling van planten aan ozon. De milieunorm is de ozonconcentratie waarbij de EC10 voor 5% van de soorten wordt overschreden. Daarnaast zijn op basis van actuele ozonconcentraties ruimtelijk expliciete schattingen gemaakt van de potentieel aangetaste fractie (PAF) van plantensoorten in Noordwest-Europa. De resultaten tonen

aan dat de milieunormen gebaseerd op de SSDs lager zijn dan de huidige normen voor blootstelling van planten aan ozon.

In **hoofdstuk 3** zijn de SSDs voor troposferische ozon gebruikt om ruimtelijk expliciete karakterisatiefactoren (CFs) af te leiden voor schade aan terrestrische vegetatie door ozonblootstelling, die is veroorzaakt door de uitstoot van antropogene stikstofoxiden (NOx) en niet-methaan vluchtige organische stoffen (NMVOCs). Deze CFs zijn voor 65 Europese regio's bepaald en worden gedefinieerd als de verandering van de PAF voor boom- en graslandsoorten als gevolg van een verandering in de uitstoot van NOx en NMVOCs. Elke CF bestaat uit een fate factor, die de relatie tussen de emissie van de precursorstoffen en de blootstelling aan ozon kwantificeert, en een effect factor, die de relatie tussen blootstelling aan ozon en de schade aan vegetatie kwantificeert. De CFs voor NOx zijn gemiddeld 3,5 keer groter dan de CFs voor NMVOCs. De CFs zijn het hoogste in Zuid-Europese regio's voor zowel NOx en NMVOCs. Verder dragen de fate en effect factor ongeveer gelijk bij aan de ruimtelijke verschillen in de CFs.

In **hoofdstuk 4** zijn drie methoden voor het afleiden van SSDs op basis van veldgegevens (f-SSDs) vergeleken door gebruik te maken van een dataset met veldwaarnemingen van graslandvegetatie en gemeten zuurgraad van de bodem (pH) op 4412 locaties in Nederland. Eén van de methoden maakt een schatting van de (maximaal) gerealiseerde soortenrijkdom langs de pH-gradiënt, terwijl de andere twee methodes de potentiële soortenrijkdom schatten. De resultaten laten zien dat potentiële soortenrijkdom van graslanden gevoeliger is voor verzuring dan de gerealiseerde soortenrijkdom. Verschillen in milieunormen voor verzuring, afgeleid met de drie f-SSD-methoden, zijn klein in vergelijking met de invloed van pH-achtergrondconcentratie op de norm. Deze resultaten geven aan dat natuurlijke achtergrondconcentraties belangrijker zijn in het afleiden van milieunormen voor pH dan het gebruik van de verschillende f-SSD-methodes.

In **hoofdstuk 5** wordt een methode gepresenteerd voor het afleiden van milieunormen die afhankelijk zijn van andere omgevingsfactoren. Om deze benadering te illustreren, is een velddataset gebruikt met soortenrijkdom van graslanden en bossen en bijbehorende metingen van de nitraatconcentratie (NO_3) en pH van de bodem verzameld op 981 locaties in Nederland. Soortenrijkdom is gerelateerd aan NO_3 en pH met behulp van kwantielregressie, waarbij interactie-effecten tussen de factoren meegenomen zijn. Vervolgens zijn de regressiemodellen gebruikt om mogelijke effecten

van verschillende pH-achtergrondconcentraties op de milieunorm van NO_3 te kwantificeren. De milieunorm is gedefinieerd als de NO_3-concentratie die beschermend is voor 95% van de soortenpoel van respectievelijk graslanden en bossen. De milieunormen zijn afgeleid voor zowel de hele soortenpoel als de Rode Lijst soorten van de verschillende vegetatietypen. De milieunorm voor NO_3 varieert tussen de 1,8 en 64,6 mg/kg, waarbij de strengste norm gevonden wordt bij een hoge pH. De resultaten tonen verder aan dat Rode Lijst soorten twee keer zo gevoelig zijn voor een toename van NO_3 vergeleken met de totale soortenpoel. Deze bevindingen geven aan dat zowel de pH-achtergrondconcentraties als de Rode Lijst soorten belangrijke factoren zijn om in overweging te nemen bij het afleiden van milieunormen voor NO_3.

In **hoofdstuk 6** zijn, op basis van dezelfde dataset die gebruikt is in hoofdstuk 4, functionele en taxonomische diversiteit in graslandvegetatie gerelateerd aan de pH van de bodem met kwantielregressie. De functionele diversiteit beschrijft de verscheidenheid aan functionele eigenschappen in een plantengemeenschap. De kwantielregressiemodellen zijn vergeleken met nulmodellen die een willekeurige soortensamenstelling langs de pH-gradiënt veronderstellen. De modellen uit de kwantielregressie lieten zien dat de patronen van taxonomische en functionele diversiteit langs de pH-gradiënt tegengesteld zijn. De taxonomische diversiteit is het hoogste bij gemiddelde pH, terwijl functionele diversiteit toeneemt naar beide uiteinden van de pH-gradiënt. De gevonden patronen verschillen daarbij van de verwachtingen op basis van de nulmodellen. De tegengestelde patronen van de taxonomische en functionele diversiteit kunnen niet alleen worden verklaard door milieufiltering- en niche divergentiehypotheses. Op basis van de resultaten lijkt er ook een rol weggelegd voor niche convergentie en facilitatie tussen soorten in de plantengemeenschappen die zijn bestudeerd. Verder geven de resultaten aan dat een gelijktijdige analyse van taxonomische en functionele rijkdom kan helpen om ecologische processen in relatie tot omgevingsfactoren beter te begrijpen.

In **Hoofdstuk 7** wordt een beschrijving gegeven van de toepassingsgebieden van de SSDs die zijn ontwikkeld in dit proefschrift, door de specifieke doelstellingen, zoals geformuleerd in hoofdstuk 1, te bespreken. De conclusie is dat de f-SSD-benadering geschikt is om een representatieve soortenpoel, meer milieufactoren en nieuwe eindpunten op gemeenschapsniveau mee te nemen. SSDs gebaseerd op laboratoriumgegevens kunnen voornamelijk worden gebruikt voor het afleiden van milieunormen voor nieuwe, giftige stoffen, terwijl f-SSDs voornamelijk kunnen worden

gebruikt in milieurisicobeoordeling, levenscyclusanalyse en biogeografisch onderzoek. Op voorwaarde dat er genoeg veldgegevens beschikbaar zijn, hebben f-SSDs de voorkeur omdat ze gemakkelijker achtergrondconcentraties en verstorende omgevingsfactoren kunnen meenemen. Verder kan met behulp van f-SSDs gemakkelijker de gevoeligheid van specifieke soortgroepen worden beoordeeld. Hoewel de resultaten in dit proefschrift laten zien dat het belangrijk is om achtergrondomstandigheden, verstorende omgevingsfactoren en specifieke soortgroepen mee te nemen, moet er nog nader worden onderzocht hoe gedetailleerd een f-SSD moet zijn.

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About the author

Curriculum vitae

On August 24th 1986 I was born in Hulst, Zeeuws-Vlaanderen. I grew up in a small village, Koewacht, where I also received primary education ('De Eikelaar'). After attending high school in Hulst ('Reynaert College'), I moved to Nijmegen to study Biology at the Radboud University in 2004. For my master's degree I spent 2 months in South Africa for a research internship at EMG and worked on a phytosociological research project at Alterra in Wageningen. In 2011 I obtained the master's degree in Biology ('cum laude') with a specialization in vegetation science.

After finishing my studies, I started working at the Raboud University Nijmegen (Department of Environmental Science) in 2012. As junior researcher, I worked for the LC-IMPACT project on modeling the effects of tropospheric ozone on terrestrial vegetation. Eventually, I had the opportunity to expand the ozone work towards a full PhD thesis. Besides my work in Nijmegen, I started in 2013 as a junior researcher at the University of Utrecht (Department of Economic and Social History). There I worked on developing an interdisciplinary database for research on historical human-nature relationships (the ATHENA project). As of January 1st 2016, I will start working as post-doctoral researcher on the ATHENA project at the Department of Environmental Science in Nijmegen.

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